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Intraspecific Variation in Salt Tolerance in Panicum Hemitomon, Spartina Patens, and Spartina Alterniflora: Population Differentiation and Investigations of Underlying Factors.

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INTRASPECIFIC VARIATION IN SALT TOLERANCE IN PANICUM HEMITOMON, SPARTINA PATENS, AND SPARTINA ALTERNIFLORA: POPULATION DIFFERENTIATION AND INVESTIGATIONS OF UNDERLYING FACTORS

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To my wife, Mary, and to the wetlands,

my life would be unimaginable in the absence of either

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Abstract

Although it is known that wetland plant species exhibit considerable interspecific variation in salt tolerance across coastal plant communities, very little is known concerning the amount of intraspecific variation in salt tolerance within plant species. Panicum hemitomon, Spartina patens and Spartina alterniflora are dominant emergent macrophytes of fresh, brackish and salt marshes, respectively. To investigate intraspecific variation in salt tolerance, plant material was collected from Gulf Coast populations of each of these species and subjected to a salinity screening protocol.

All three of the plant species displayed significant intraspecific variation in lethal salinity level and plant morphology. Lethal salinity levels ranged from 7.6‰ to 12.0‰ in Panicum hemitomon, from 63‰ to 93‰ in Spartina patens, and from 83‰ to 115‰ in Spartina alterniflora. Population morphological differences were most correlated with salt tolerance in Panicum hemitomon, the fresh marsh dominant and least correlated with Spartina alterniflora, the salt marsh dominant.

Investigations conducted at sublethal salinity levels on subsets of populations showed that plant photosynthetic response was able to differentiate highly salt-tolerant and poorly salt-tolerant populations within each species to varying degrees. These differences were greatest in Panicum populations, with the highly salt-tolerant populations having higher photosynthetic rates and greater water use efficiencies. Highly salt-tolerant populations of Panicum hemitomon and Spartina patens were able to limit the total cation concentrations in their leaves, maintain greater leaf xylem pressures, and accumulate less proline than poorly salt tolerant populations, but apparently had only limited control over the ionic composition. Conversely, Spartina alterniflora populations showed no differences in leaf total cation concentrations, but the highly salt-tolerant populations were able to selectively decrease their Na:K ratio and accumulate more glycinebetaine than poorly salt-tolerant populations.

It is concluded that in Panicum hemitomon plant size factors and photosynthetic rates are important in explaining population differences in salt tolerance by providing more mature tissue for the translocation of salts away from actively growing regions. The importance of plant morphology decreases as physiological/biochemical responses become progressively more important in explaining intraspecific variation in salt tolerance in Spartina patens and Spartina alterniflora.

Chapter 1

Introduction

Wetland loss is of global concern. The deterioration of wetlands is generally first expressed in the stress and death of wetland vegetation, followed by subsequent erosion and wetland loss. Elevated salinity level is one of the potential causes of stress and ultimate death of wetland vegetation and, therefore, has been implicated as a factor affecting wetland loss (Boesch 1982; Mendelssohn et al. 1983; Salinas et al. 1986; Turner and Cahoon 1987). Increases in salinity regime may generally be attributed to sea level rise, canalization, and storm surges associated with hurricanes and severe storm events (Turner et al. 1982; Salinas et al. 1986). Other alterations of hydrology, such as the abandonment of a freshwater tributary, may lead to increased salinities in the surrounding marshes (Fisk 1955; Penland et al. 1981).

It is well recognized that species differences in salinity tolerance (interspecific variation in salt tolerance) are largely responsible for the broad zonation of coastal plant communities into what has been classified as fresh, intermediate, brackish and salt marsh plant communities located along the gradient from fresh to saline water (Chabreck 1972; Pomeroy and Wiegert 1981; Mitch and Gosselink 1986; Day et al. 1989). In infrequently flooded coastal areas, such as high marsh areas and salt pans where salts accumulate to high levels at the soil surface as water evaporates from the soil, interspecific variation in salt tolerance can also be important in explaining small-scale vegetation patterns and dynamics associated with environmental heterogeneity in salinity (Bertness 1991; Shumway and Bertness 1994). However, the relative importance and extent of intraspecific variation in salt tolerance in coastal plant communities has not been intensively investigated and may be important in understanding how these plant

communities respond to changes in salinity regime, whether caused by natural or anthropogenic factors.

Panicum hemitomon, Spartina patens, and Spartina alterniflora are dominant emergent macrophytes of the fresh, brackish, and salt marsh plant communities, respectively. Panicum hemitomon Schult. is a fresh marsh grass that is distributed continuously along the coastal plain of the United States from New Jersey southward into Florida and westward along the Gulf coast into Texas. It is also found in some fresh marshes in Tennessee, as well as in South America (Godfrey and Wooten 1979). Throughout its range Panicum hemitomon is a common species in the fresh marsh plant community and in Louisiana it is the dominant emergent macrophyte of the State's southeastern coastal freshwater marshes (Chabreck 1972).

Spartina patens (Ait.) Muhl. is a very important coastal grass species that is distributed continuously along the Atlantic and Gulf coasts of North America from Maine to south Texas. Spartina patens also occurs in the Yucatan peninsula, Cuba, the West Indies, and along some sandy shores of the Great Lakes (Mobberly 1956; Godfrey and Wooten 1979). Spartina patens has a wide ecological amplitude, ranging from dunes and swales to coastal intermediate and brackish marshes, where it is usually dominant (Duncan and Duncan 1987). In Louisiana, Spartina patens is the dominant emergent macrophyte in the State's expansive brackish marshes and is the most frequently encountered grass species throughout the coastal zone (Chabreck 1970; 1972).

Spartina alterniflora Loisel. has been referred to as the most important grass species in coastal salt marshes (Pomeroy and Wiegert 1981; Duncan and Duncan 1987; Eleuterius 1990). Spartina alterniflora is distributed continuously along the Atlantic coast from New Foundland southward to Florida and westward along the Gulf coast to Texas (Godfrey and Wooten 1979). Spartina alterniflora is generally the dominant coastal salt marsh grass throughout its range, and in Louisiana it dominates the vast southern expanses of the State's coastal salt marshes (Chabreck 1972).

Although it is well known that fresh, brackish and salt marsh plant species exhibit considerable interspecific variation in terms of salt tolerance, very little is known concerning the amount of variation in salt tolerance within each species (intraspecific variation). The work of Silander (1979) and Silander and Antonovics (1979) on Spartina patens represent the most extensive research to date on intraspecific variation in salt tolerance in a coastal grass species. Their research on dune, swale and marsh ecotypes of North Carolina populations of Spartina patens revealed that populations growing in these different coastal habitats were often genetically distinct and had different rates of root growth under saline conditions. Recently, Pezeshki and DeLaune (1991; 1995) have shown that population differentiation in salinity tolerance may exist in Louisiana marsh populations of Spartina patens and Spartina alterniflora. Although their research was based on a limited comparison of two populations within each species located along the extremes of a natural salinity gradient, their research nonetheless has shown that population differentiation to salinity tolerance may exist in these two species. However, none of the studies to date have looked at intraspecific variation in salinity tolerance in a large number of Gulf Coast populations of Spartina patens or Spartina alterniflora, and to my knowledge there have been no published investigations on intraspecific variation in salinity tolerance in Panicum hemitomon.

Decreased growth of plants subjected to increased soil salinity can occur primarily for three reasons: 1) a water deficit due to a low external water potential, 2) an excess of ions in the tissue due to uptake of salts from the soil solution, and 3) an inhibition of nutrient ion uptake due to excessive Na^+ or Cl^- concentrations (Gorham et al. 1985). Halophytes (salt-tolerant plant species, such as Spartina alterniflora and Spartina patens) are able to accumulate high concentrations of salts in their tissues for osmotic adjustment through the compartmentalization of ions in vacuoles and the production of neutral organic solutes in the cytoplasm. These neutral organic solutes maintain a low cytoplasm osmotic potential, such that water movement can still occur down a water potential

gradient into the living cells (Gorham et al. 1985). Some neutral organic solutes that may show an increase in concentration under salinity stress, and thus may function in osmotic adjustment, include proline, glycinebetaine and sugars (Storey and Wyn Jones 1978; Briens and Larher 1982; Cavalieri and Huang 1979, 1981). It has also been reported that proline may protect enzymes (proteins) from dehydration (Paleg et al. 1985) or hydroxyl radicals (Smirnoff and Cumbes 1989). Regardless of its function, significant proline accumulation appears to occur once a threshold of drought or salt stress is exceeded (Cavalieri and Huang 1979) and, therefore, may prove useful in differentiating populations for salinity tolerance.

Halophytes may also control tissue Na^+ or Cl^- concentrations through exclusion at the root, control of translocation of ions to shoots, and/or secretion from the leaves through salt glands (Flowers 1985). Spartina alterniflora and Spartina patens (salt marsh and brackish marsh dominants, respectively) both possess the ability to secrete salts out of the plant and onto the leaf surfaces through salt glands (Anderson 1974; Bradley and Morris 1991). Spartina alterniflora is also able to exclude salt (Smart and Barko 1980) accumulate it in the leaf (Nestler 1977), and exert some selectivity over which ions accumulate in its tissues (Bradley and Morris 1991). These mechanisms allow these two species to effectively control tissue electrolyte concentrations under conditions typically encountered in the field. In addition, these two Spartina species are known to accumulate compatible organic solutes, such as proline and glycinebetaine, that may aid in osmotic adjustment (Cavalieri and Huang 1979, 1981).

Glycophytes (nonhalophytes, such as Panicum hemitomon, a fresh marsh dominant) usually fall into two major categories of response to salinity: 1) those that cannot avoid ion uptake and experience adverse effects when electrolyte concentrations become excessive and 2) those that avoid ion uptake (Greenway and Munns 1980). Glycophytes that exclude ions must adjust osmotically by producing compatible organic solutes to avoid a water deficit, although the cost of producing sufficient compatible

solutes generally limits this strategy to relatively low external salt concentrations (Yeo 1983). Panicum hemitomon does accumulate Na^+ in its shoots when grown at elevated salinity levels (McKee and Mendelssohn 1989). Although Na^+ accumulation may contribute to osmotic adjustment, this accumulation apparently becomes excessive and severely inhibits growth once salinity levels exceed 9‰ (McKee and Mendelssohn 1989).

In addition to responses in terms of ionic relations or compatible solute production, there are also morphological characteristics that may be associated with salinity tolerance. Since a primary effect of salt stress is a water deficit, characteristics such as leaf morphology may be associated with transpirational water loss. Wetland plants grown under saline conditions often develop smaller leaves or a shorter growth form (Shea et al. 1975; Cain and Harvey 1983; Eleuterius 1989). Whether plant populations of dominant wetland species display morphologic differentiation that may be associated with ultimate salt tolerance is not known. Leaf rolling can occur as a response to decreased plant water status (Begg 1980) and has been utilized in screening agricultural plants for drought resistance (O'Toole and Cruz 1980). Therefore, leaf rolling response may provide a means of differentiating wetland plants in terms of salinity tolerance.

The goal of this study was to determine whether geographically distinct Gulf Coast populations of Panicum hemitomon, Spartina patens and Spartina alterniflora display significant intraspecific variation in salt tolerance and then to investigate whether certain key morphological, physiological, and biochemical characteristics are associated with degree of salt tolerance. Through this series of investigations it was hoped that a greater understanding of population differentiation and mechanisms of salinity tolerance in these important coastal grass species would be achieved, and that the information obtained may facilitate future screenings of wetland vegetation for superior salt tolerance.

To accomplish this goal, a two-phase research plan was designed. In the first phase, Gulf Coast plant populations of Panicum hemitomon, Spartina patens and

Spartina alterniflora were screened for intraspecific variation in salt tolerance. In the second phase, a subset of populations from each species, that ranged from highly salt tolerant to poorly salt tolerant, were subjected to a sublethal salinity excursion and plant growth responses and physiological/biochemical response were determined in control and salt-challenged treatments.

In Chapter 2, the first research chapter, intraspecific variation in salt tolerance and plant morphology was investigated in Spartina patens. Nineteen populations of Spartina patens were collected throughout the Gulf Coast marshes of Louisiana, Texas and Florida. These populations were de-acclimated from the varying field salinity conditions for several vegetative generations and a number of plant morphological variables were measured. The Spartina patens populations were then subjected to a lethal salinity excursion. Under controlled conditions, salinity was increased in weekly stepwise increments until 50% death of aboveground tissue was observed, which was defined as the lethal salinity level. Plant leaf expansion rates and leaf rolling indices were measured during the salinity excursion and at harvest, plant biomass was determined. The results from this experiment provide insight into intraspecific variation in salt tolerance and plant morphology in this widely distributed coastal grass species.

Intraspecific variation in salt tolerance and plant morphology in Panicum hemitomon and Spartina alterniflora was investigated in Chapter 3. The experimental design for the lethal salinity excursions of these two species is similar to that presented for Spartina patens. The results from these two experiments are combined into one chapter to provide insight into the similarities and differences in intraspecific variation in plant morphology and salt tolerance when a fresh marsh dominant and a salt marsh dominant are subjected to lethal salinity excursions.

In the next three research chapters, Chapters 4, 5 and 6, results are presented by topic from the three separate species experiments in the second phase of these investigations. In these investigations, subsets of populations within each species were

selected to range from highly salt tolerant to poorly salt tolerant and then subjected to a sublethal salinity excursion. For each species, plant growth responses and physiological/biochemical responses were determined at an early harvest (one week after reaching the sublethal salinity level) and at a late harvest (five weeks after exposure to the sublethal salinity). Chapter 4 presents the results from these experiments on plant photosynthesis and growth response in the first part of what is written as a three part series entitled Investigations of Factors Associated with Intraspecific Variation in Salt Tolerance in Panicum hemitomon, Spartina patens and Spartina alterniflora.

In Chapter 5, the second part of this three-part series, results are presented on species and population differences in biomass partitioning. The results from this chapter provide insight into species differences and population differences within species in terms of how plant biomass is partitioned in control and salt-challenged populations of known varying degree of salt tolerance.

The third manuscript of this three-part series is presented in Chapter 6. This final research chapter presents results on leaf water potential, proline, glycinebetaine and leaf cation concentration in each of the three species under controlled and sublethal salinity levels. The information derived from these investigations provides a better understanding of species differences in salt tolerance response, and also of intraspecific differences within species in tolerating a salinity stress event.

Chapter 7 is the conclusion chapter of the dissertation. This chapter summarizes the main findings of all the research chapters and discusses what overall conclusions can be drawn from these investigation, as well as possible future research directions.

The research chapters are written in journal format for publication in appropriate journals. As a result of being written to basically stand alone, there is some redundancy in the chapter introductions and methods.

Chapter 2

Intraspecific Variation in Salt Tolerance and Morphology in the Coastal Grass Spartina patens (Poaceae)

INTRODUCTION

One of the potential causes of stress and ultimate death of wetland vegetation is increased salinity, which may result from canalization, storm surges associated with severe storm events or hurricanes, and sea level rise (Turner et al. 1982; Mendelssohn et al. 1983; Salinas et al. 1986). Although it is well known that wetland plant species exhibit considerable interspecific variation in salt tolerance across coastal plant communities (Chabreck 1971; 1981), very little is known concerning the amount of variation in salt tolerance within a species. Spartina patens is an important coastal grass species distributed continuously along the Atlantic and Gulf coasts of North America from Maine to south Texas. It is also found in the Yucatan peninsula, Cuba, the West Indies, and along some sandy shores of the Great Lakes (Mobberly 1956). Spartina patens has a wide ecological amplitude, ranging from barrier island dunes and swales to coastal brackish and intermediate salinity marshes. In Louisiana coastal marshes, Spartina patens is the dominant emergent macrophyte in the brackish marsh plant community, where soil salinities typically range from 2.0‰ (ppt, i.e., g salt kg^{-1} solution) up to 15.2‰, where it is replaced by Spartina alterniflora (Chabreck 1970; 1972). Spartina patens remains an important species in the intermediate marshes where soil salinities range from 1.5‰ to 5.3‰ (Chabreck 1970; 1972).

Silander (1979) and Silander and Antonovics (1979) documented adaptive genetic divergence in dune, swale, and marsh subpopulations of Spartina patens and reported that these subpopulations differed in an index of salt tolerance based on root elongation in a saline solution. Pezeshki and DeLaune (1991) reported that Spartina patens collected from a brackish-saltwater population grew better at elevated salinities

than Spartina patens collected from a lower salinity fresh-water-brackish population. However, none of the studies to date have investigated intraspecific variation in salt tolerance across a large geographical area, nor has an attempt been made to quantify salt tolerance in terms of actual lethal salinity level.

The primary objective of this study was to determine whether geographically distinct stands (hereafter referred to as populations) of Spartina patens display significant intraspecific variation in salinity tolerance. A secondary objective was to determine whether these populations display intraspecific variation in plant morphology, leaf rolling and growth rates. If significant intraspecific variation was identified in salinity tolerance, plant morphology, leaf rolling and growth rates, a final objective was to investigate whether a correlation exists between salinity tolerance and these other plant traits and responses. To accomplish these objectives, populations of Spartina patens were subjected to weekly stepwise increases in salinity until 50% death of aboveground tissue was observed. Plant morphological variables were measured prior to salinity increase, and plant growth rates and leaf rolling were measured at several sublethal salinity levels during the salinity excursion. Above- and belowground biomass were determined at harvest.

MATERIALS AND METHODS

Plant material

Clones from 19 geographically distinct populations of S. patens were collected from coastal marshes of Texas (12 clones), Florida (two clones) and Louisiana (five clones). The US Soil Conservation Service Plant Materials Laboratory assisted by providing us with some of these collections. Field collection sites covered a wide range of salinities and included low salinity intermediate marshes, brackish marshes, brackish/saline marshes, and irregularly flooded brackish/saline marshes that bordered salt pans. Single stems from each population were vegetatively propagated for three generations under uniform, non-saline conditions in the greenhouse over a one year

period to reduce any field acclimation to varying salinity conditions and to ensure that each population was represented by a single genotype. Propagation was accomplished by physically separating new tillers (ramets) from the parent ramet, discarding the parent and then repotting the young ramets. This process was repeated three times with the fourth generation ramets used for the experiment. Plants were propagated in a commercial potting mix (Jiffy Mix®; Chicago Illinois) and all pots were given equal amounts of Hoagland's nutrient solution (Hoagland and Arnon 1950) as needed to produce vigorous growth prior to potting in the experiment.

Experimental design

The salinity screening experiment was set up as a randomized block design of five replicates in a glasshouse on the LSU, Baton Rouge, Louisiana campus. Depending on the size of the plants, four to six young stems were planted per pot to minimize weight differences in the amount of initial plant material per experimental unit. Initial plant wet weight was also recorded and tested for statistical significance as a covariable for harvest biomass measurements. Five replicates of each of the 19 populations were potted in Jiffy Mix in 0.5 liter plastic pots equipped with bottom and side drainage holes. These pots were placed inside larger reservoir pots that contained the treatment bathing solutions of increasing salinity through time. Treatment bathing solutions were maintained at a level of 4 to 5 cm below the soil surface. This allowed exchange of soil interstitial water with the treatment bathing solution without flooding the soil surface. The plants were established in April and the experiment ran through August. The midday illumination (photon flux density) inside the glasshouse ranged from 1100 to 1400 μmoles m^{-2} s^{-1}. Average maximum and minimum temperatures inside the glasshouse were 35 C and 24 C, respectively, with an average midday relative humidity of 60%.

Salinity regime

After transplanting, all experimental units were maintained in a treatment bathing solution of full strength Hoagland's nutrient solution at 0‰ salinity for 10 days. Salinity was then increased to 2‰ in full strength Hoagland's for 10 days using a commercial synthetic sea salts mix (Instant Ocean®; Aquarium Systems, Inc., Mentor, Ohio) with major ionic composition expressed as percentage of dry weight as follows: 46.9% Cl, 26.0% Na, 6.4% SO_4, 3.2% Mg, 1.0% Ca, and 0.9% K (Bidwell and Spotte 1985). Salinity was then increased to 5‰ for one week. Thereafter, salinities were increased weekly in 5‰ increments until all experimental units reached their lethal salinity level, which we defined as the salinity level resulting in 50% death of aboveground tissue. All salinity increases throughout the experiment followed the following procedure: 1) Pots were removed from reservoir containers and allowed to drain for one hour. 2) Pots were then flushed with two 150 ml aliquots of Instant Ocean solution at 2‰ higher salinity than the targeted salinity by slowly adding the solution to the soil surface and allowing it to drain through the soil and out the drainage holes over a period of two hours. 3) Pots were placed back inside the reservoir containers. 4) A solution of full strength Hoagland's with Instant Ocean at the targeted salinity was added to the soil surface in 150 ml and 175 ml aliquots, which drained through the soil and collected in the reservoir containers, bringing the solution in the reservoir up to the proper level. Salinity was rechecked for accuracy. Solution levels and salinities were checked daily.

Variables measured

The following plant morphological variables were measured on two randomly selected stems from a pool of representative stems in each pot immediately prior to salinity increase: plant height (to the top ligule, i.e. to the ligule of the youngest expanded leaf; to the tip of the emerging terminal leaf; and maximum height to the tallest vertically straightened leaf tip), leaf length and width (on the top two expanded leaves below the terminal leaf, hereafter referred to as leaf one and leaf two), internode distance

between leaves one and two, stem diameter measured between leaves one and two, number of expanded green leaves per stem, and total number of expanded leaves per stem.

Leaf expansion measurements were made at salt concentrations of 2‰, 20‰, 30‰, 40‰, and 65‰. Leaf expansion was measured on randomly selected stems from a pool of stems having terminal leaves that were neither newly emergent nor fully expanded, but in the range of one third to two thirds expanded. Leaf length was measured from the tip of the terminal leaf to the ligule of the youngest expanded leaf over a time interval of three days. This method has been used successfully with other grasses and produced results which parallel changes in aboveground biomass and photosynthesis (Hester and Mendelssohn 1990).

Other studies have shown that leaf rolling may be an indicator of plant water stress that can be utilized in screening crop plants for drought tolerance and, hence, may be of value in screening for salt tolerance (O'Toole and Moya 1978; O'Toole and Cruz 1980). Leaf rolling was measured on the top two expanded leaves of the stems tagged for leaf expansion measurements. A leaf rolling index was calculated at the midpoint of the leaf as the projected width of the leaf divided by the unrolled width of the leaf (Begg 1980). Therefore, a leaf that is not rolled will have an index of 1.0, whereas leaves that are rolled will have indices less than 1.0. A severely rolled leaf of S. patens will have an index in the range of 0.4 to 0.6. Leaf rolling measurements were taken at all salinity levels between 2‰ and 65‰.

Plants were harvested when an experimental unit reached its lethal salinity level (comparable to an LD_{50}) as determined by visually assessing the relative amounts of live (green) and dead (brown) aboveground tissue. Once a greater proportion of brown tissue was observed, the experimental unit was harvested. Aboveground tissue was lightly rinsed with tap water to remove any encrusted salts on the leaf surfaces. Soil was rinsed from the roots, and the plant was partitioned into aboveground and belowground

components. After blotting the tissue to remove excess moisture, aboveground and belowground wet weights were determined. Samples were oven dried at 65 C for five days until constant weight was achieved and dry weight determined.

Data analysis

Data were analyzed as a randomized block design. Analysis of variance (ANOVA) was used to test for significant population differences using SAS (SAS 1989; Steel and Torrie 1980). A significance (alpha) level of 0.05 was used for all analyses unless otherwise stated. All data were tested for meeting the assumptions of normality (Shapiro-Wilk test statistic) and homogeneity of variance (Bartlett test statistic) using SAS (SAS 1989). Belowground biomass data were log-transformed to meet these assumptions. Leaf rolling indices could not be suitably transformed, so non-parametric statistics were employed. Population differences in leaf rolling indices were tested by ranking the data within blocks followed by a randomized block design ANOVA (Friedman two-way ANOVA; Siegel and Castellan 1988).

Correlation matrices were constructed to investigate linear relationships between plant morphological variables, growth rates, and leaf rolling indices with lethal salinity level and covariable-adjusted total plant dry weight. Correlations were conducted at two levels: 1) at the level of the individual based on all experimental units after removing the effect of block, since significant block effects were detected in all variables (n=95), and 2) at the population level based on population means (n=19). Conducting correlations on population (or treatment) means is recommended when the level of interest is at the population (or treatment) level to avoid confounding the residual error with variation between experimental units of the same population (i.e. experimental error; Gomez and Gomez 1984). Correlations with leaf rolling indices were done using Spearman correlations (non-parametric), whereas other correlations employed Pearson correlations.

Principal components analysis (PCA) was used 1) to investigate intraspecific variation expressed in a created variable based on positive loadings of two desirable

salinity tolerance traits: plant production under increasing salinity stress and lethal salinity level, 2) to investigate relationships in the principal component loadings of lethal salinity level and morphological traits, and 3) to obtain principal component scores based solely on plant morphological data that were outputted and correlated with salt tolerance (Johnson and Wichern 1988). In all of these applications PCA was run on residuals that were standardized to a mean of zero and a variance of one after the effect of block was removed. Standardization of the residuals prior to principal components analysis was done to give all variables equal weight since the units of measure of the variables differed considerably.

RESULTS

Salt Tolerance

Highly significant ($P<0.01$) differences were found among populations in lethal salinity level (salinity that resulted in 50% death of aboveground tissue). Lethal salinity levels ranged from a low tolerance of 63.0‰ to a high tolerance of 93.0‰ (Figure 2.1 top panel). The majority of the populations had lethal salinity levels between 80‰ - 83‰ (Figure 2.1 top panel).

Highly significant ($P<0.01$) differences among populations were detected in wet and dry aboveground, belowground, and total biomass (Table 2.1). Wet weight data followed the same trends as dry weight data and are not presented. Aboveground dry weight ranged from 7.2 to 31.0 g per pot with belowground dry weight ranging from 1.2 to 8.6 g per pot (Table 2.1). Total plant dry weight ranged from 8.5 to 39.5 g per pot (Table 2.1). Belowground-to-aboveground dry weight ratios also displayed highly significant differences among populations and ranged from 0.133 to 0.349 (Table 2.1). The covariable, initial plant wet weight at the time of planting, showed significant differences between populations despite efforts to minimize this effect. Therefore, only covariable-adjusted total plant dry weight is used throughout the remainder of the

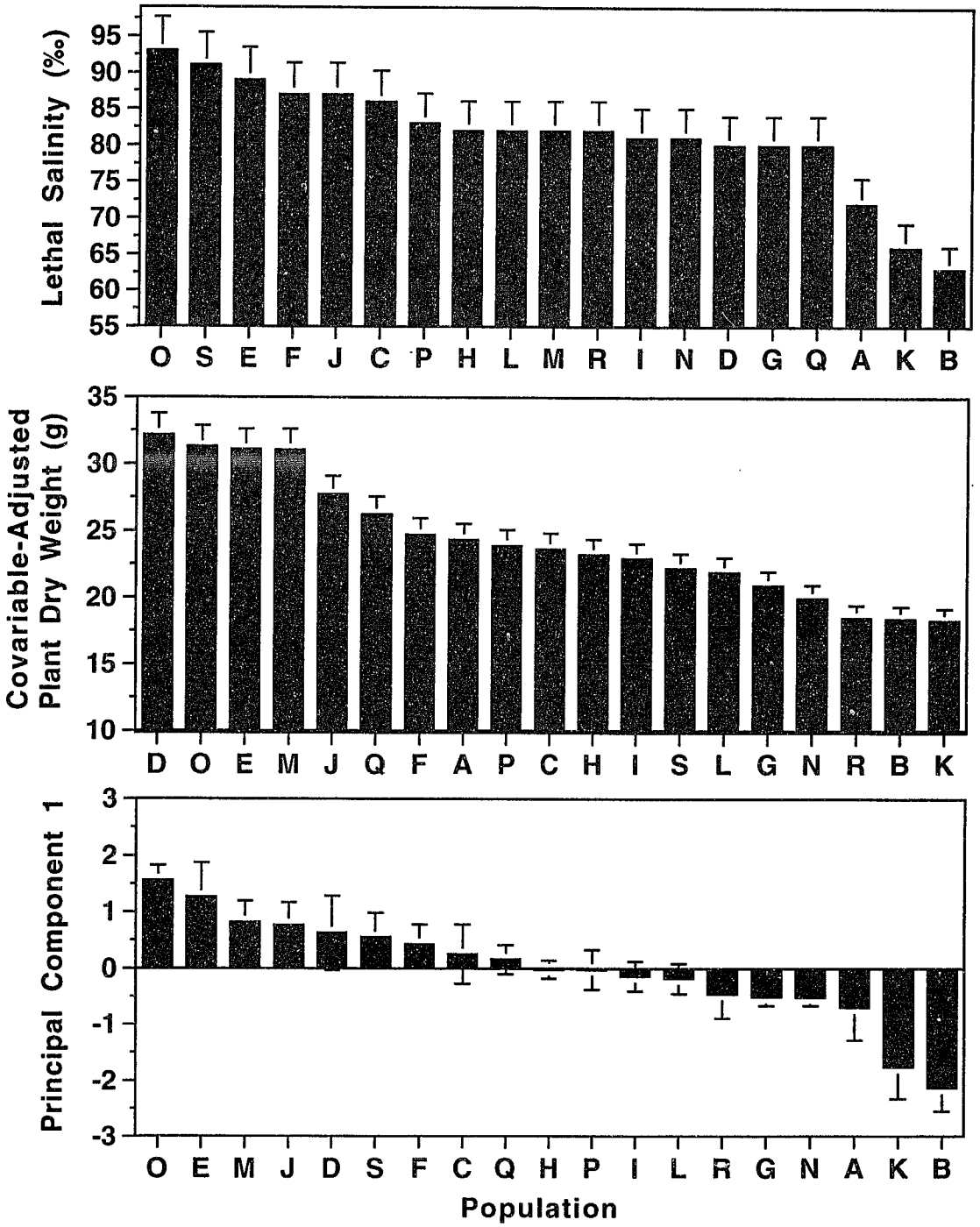

Figure 2.1. Lethal salinity level (salinity resulting in 50% death of aboveground tissue; top panel; $LSD_{0.05}$=7.71; MSE=37.35), covariable-adjusted total plant dry weight (middle panel, $LSD_{0.05}$=7.48, MSE=35.17), and principal component one scores (bottom panel, $LSD_{0.05}$=1.15, MSE=0.84) from a principal components analysis of lethal salinity level and covariable-adjusted total plant dry weight for 19 populations of Spartina patens (n=5).

Table 2.1. Range of population dry-weight means (n=5) for plant biomass parameters in 19 populations of Spartina patens harvested at their respective lethal salinity levels, the resultant F values (18, 72 df), and probability levels of a significant population difference. Biomass is expressed as g per pot.

	Range			
Variable	Minimum	Maximum	F value	Probability > F
Aboveground	7.2	31.0	4.74	0.0001
Belowground	1.2	8.6	9.80	0.0001
Total	8.5	39.5	5.12	0.0001
Total Covariable-Adjusted	18.4	32.1	2.79	0.0011
Belowground to Aboveground Ratio	0.133	0.349	6.65	0.0001

manuscript. Biomass rankings based on covariable-adjusted total plant dry weight are presented in Figure 2.1 (middle panel) and show a range from 18.4 to 32.1 g per pot.

A principal components analysis of lethal salinity level and covariable-adjusted total plant dry weight was conducted to investigate intraspecific variation expressed as a created variable that combined these two desirable traits. Figure 2.1 shows the relationship between lethal salinity level (top panel), covariable-adjusted plant dry weight (middle panel) and the first principal component (bottom panel), which had high positive loadings of both traits and was able to explain 74% of the variation among populations. An ANOVA of the outputted principal component 1 scores revealed highly significant population differences. Based on this analysis, populations with high principal component 1 scores are classified as highly salt tolerant, characterized by high production under salinity stress and also high lethal salinity level. Populations with low scores are classified as poorly salt tolerant, characterized by low production under salinity stress and a low lethal salinity level.

All populations showed a trend of decreasing leaf expansion rate with increasing salinity level (Figure 2.2). Significant population differences in leaf expansion rates were detected at 2‰ and 20‰. As salinities increased, the populations converged toward more similar, lower expansion rates (Figure 2.2). At salinities of 30‰ and 40‰ population differences were significant only at $P<0.10$, and at 65‰ were no longer significantly different. Populations did not maintain a consistent ranking of leaf expansion rates across increasing salinities.

There were highly significant ($P<0.01$) differences among populations in the extent to which leaves were rolled at ten of the eleven salinity levels measured. At 5‰ and 10‰ only a few populations displayed leaf rolling, but this resulted in highly significant differences among populations. At 15‰ none of the populations displayed leaf rolling. Beginning at 20‰ and continuing for every 5‰ salinity increase up to 55‰, populations displayed highly significant differences in leaf rolling. Figure 3

shows leaf rolling on a subset of six populations ranging from highly salt tolerant to poorly salt tolerant. Although this subset does not reflect the differences observed in some populations at the lower salinities, the trend of leaves to be more tightly rolled at salinities greater than 30‰ is readily observed. Also, many populations displayed leaf rolling at 20‰ - 25‰ and then relaxed this response slightly at 30‰ before becoming more tightly rolled as salinity increased above 30‰ (Figure 2.3). Responses were quite variable and populations did not maintain a consistent ranking of leaf rolling indices across increasing salinities (Figure 2.3).

Plant morphology

Highly significant ($P<0.01$) differences among populations were found in all the plant morphological variables measured prior to salinity stress. Table 2.2 shows the range of population differences in morphological variables and also lists the F values from tests of population effects, thereby providing information on the relative strength of population differences in morphology (i.e., larger F values indicate stronger population differences). Plant height to the upper ligule ranged from 34 to 67 cm, with maximum plant height ranging from 88 to 119 cm (Table 2.2). The number of leaves per stem ranged from 4.0 to 6.8 (Table 2.2). Leaf morphology showed an especially large amount of intraspecific variation with leaf widths ranging from 0.30 to 0.56 cm ($F=15.47$) and leaf lengths ranging from 25.2 to 62.0 cm (Table 2.2). Leaf length x width was also highly variable among populations and displayed a three-fold range of population differences from 10 to 30 cm^2 (Table 2.2).

Correlations with salt tolerance and plant production

Leaf expansion rates at 20‰ and 40‰ were significantly correlated with lethal salinity level (Table 2.3). However, the strength of these correlations were fairly weak since the respective r^2 values explained only 6% and 8% of the variation in lethal salinity level among individual experimental units and were not significant when based on population means. Leaf expansion rates of individual experimental units at 20‰ and

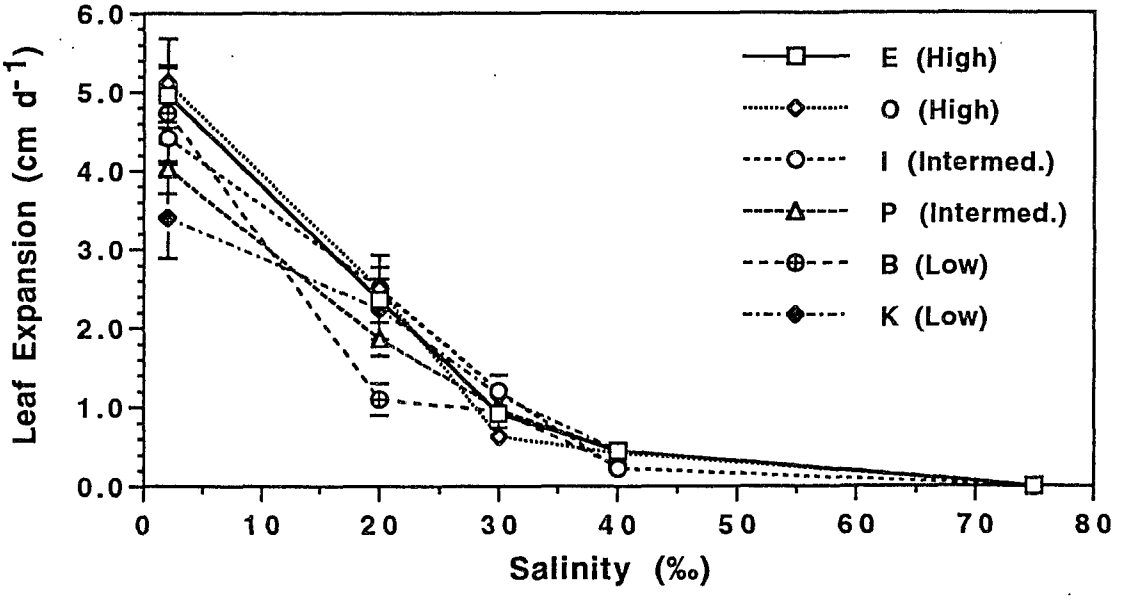

Figure 2.2. Leaf expansion rate for a subset of 6 of the 19 populations of Spartina patens as salinity was increased in weekly increments of 5‰. Shown are two populations with a high lethal salinity level (high), two populations with an intermediate lethal salinity level (intermed.), and two populations with a low lethal salinity level (low), n=5.

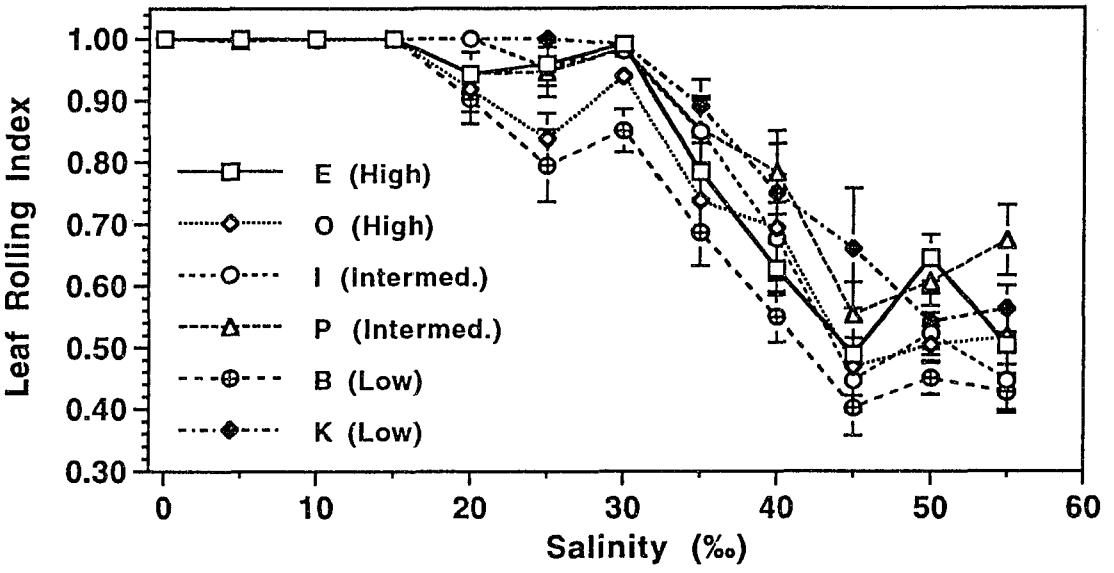

Figure 2.3. Leaf rolling index (projected leaf width/unrolled leaf width) for a subset of 6 of the 19 populations of Spartina patens as salinity was increased in weekly increments of 5‰. Shown are two populations with a high lethal salinity level (high), two populations with an intermediate lethal salinity level (intermed.), and two populations with a low lethal salinity level (low), n=5.

Table 2.2. Range of population means (n=5) for plant morphological variables in 19 populations of Spartina patens following three generations of growth under non-saline conditions, the resultant F values (18, 72 df), and probability levels of a significant population difference.

	Range			
Variable	Minimum	Maximum	F value	Probability > F
Plant Height (cm)				
to upper ligule	33.63	66.59	4.90	0.0001
to growing tip	73.10	105.85	2.29	0.0070
to tallest leaf tip	88.12	119.12	4.30	0.0001
Leaves per Stem	4.0	6.8	3.52	0.0001
Internode Distance (cm)[a]	3.98	7.88	2.64	0.0019
Stem Diameter (cm)[a]	0.11	0.20	9.22	0.0001
Leaf				
Length (cm)[b]	25.16	61.94	6.27	0.0001
Width (cm)[b]	0.30	0.56	15.47	0.0001
Length x Width (cm^2)[b]	10.05	30.22	9.16	0.0001

[a]measured between the top two expanded leaves.
[b]measured on the top two expanded leaves.

Table 2.3. Partial and semi-partial correlation coefficients (r) and significance levels (P > | r |) for correlations of leaf expansion rates (Pearson correlations) and leaf rolling indices (Spearman correlations) at the indicated salinity levels with lethal salinity level and covariable-adjusted total plant dry weight. Significant (P<0.05) correlations are shown in bold face type (n=95). Significant correlations based on population means (n=19) are shown in bold face type within parentheses as a second row for the corresponding variable pairs.

	Lethal Salinity Level		Covariable-Adjusted Total Plant Dry Weight	
	r	Prob > | r |	r	Prob > | r |
Leaf expansion				
2‰	0.054	0.600	0.115	0.266
20‰	**0.242**	**0.018**	**0.462**	**0.0001**
			(0.548)	**(0.015)**
30‰	0.079	0.444	0.195	0.059
40‰	**0.290**	**0.004**	**0.244**	**0.017**
Leaf rolling index				
5‰	-0.013	0.898	0.032	0.761
10‰	-0.143	0.166	0.021	0.838
15‰	-0.078	0.452	-0.113	0.275
20‰	-0.176	0.088	-0.107	0.303
25‰	-0.130	0.209	-0.039	0.707
30‰	0.164	0.113	-0.001	0.986
	(0.539)	**(0.017)**		
35‰	0.054	0.605	-0.028	0.791
40‰	0.051	0.622	-0.009	0.933
45‰	0.039	0.708	0.009	0.933
50‰	0.102	0.327	-0.073	0.485
55‰	0.081	0.440	-0.128	0.222

40‰ were significantly correlated with covariable-adjusted total plant dry weight (Table 2.3). Leaf expansion at 20‰ explained 21% of the variation in covariable-adjusted total plant dry weight among experimental units and 30% of the variation among populations (Table 2.3).

None of the leaf rolling indices of individual experimental units measured between 5‰ - 55‰ yielded significant correlations with lethal salinity level or covariable-adjusted total plant dry weight (Table 2.3). At the population level, leaf rolling at 30‰ was significantly correlated with lethal salinity level and explained 29% of the variation in salt tolerance among populations (Table 2.3).

Several of the plant morphological variables measured prior to salinity increase had significant correlations with lethal salinity level, although none were significantly correlated with covariable-adjusted total plant dry weight (Table 2.4). Lethal salinity level of individual experimental units had significant positive correlations with plant height (measured to the tallest leaf tip), leaf length and leaf length x width (Table 2.4). For both individual experimental units and populations leaf length had the strongest correlations, explaining 18% of the variation in lethal salinity level among individuals ($r=0.43$) and 35% of the variation in lethal salinity level among populations ($r=0.59$; Table 2.4).

Results from the principal components analysis of lethal salinity level and eight plant morphological variables showed that 77% of the variation among populations was explained by the first three principal components (Table 2.5). The first two principal components were interpreted to be a leaf size component and a leaf number component, respectively, and explained 61% of the variation among populations (Table 2.5). Lethal salinity level had a high loading on the third principal component and was associated with a positive loading of leaf length contrasted against internode distance (i.e. long leaves with short internode distances between them). This high salinity/long leaf/short

Table 2.4. Partial and semi-partial correlation coefficients (r) and significance levels (P > | r |) for correlations of plant morphological variables prior to salinity stress and belowground to aboveground (dry weight) ratio at harvest with lethal salinity level and covariable-adjusted total plant dry weight. Significant (P<0.05) correlations are shown in bold face type (n=95). Significant correlations based on population means (n=19) are shown in bold face type within parentheses as a second row for the corresponding variable pairs.

	Lethal Salinity Level		Covariable-Adjusted Total Plant Dry Weight	
	r	Prob > | r |	r	Prob > | r |
Plant Height				
to upper ligule	-0.090	0.388	-0.099	0.338
to growing tip	0.175	0.090	-0.001	0.994
to tallest leaf tip	**0.309**	**0.002**	0.044	0.672
Leaves per Stem	-0.167	0.107	-0.049	0.635
Internode Distance	-0.076	0.463	0.011	0.918
Stem Diameter	0.116	0.263	0.090	0.385
Leaf				
Length	**0.428**	**0.0001**	0.187	0.070
	(0.590)	**(0.008)**		
Width	-0.002	0.982	0.119	0.249
Length x Width	**0.277**	**0.007**	0.183	0.076
Belowground to Aboveground Ratio	-0.049	0.635	-0.042	0.686

Table 2.5. Eigenvalues, eigenvectors, and interpretation of the first three principal components from a nine variable principal components analysis of lethal salinity level and plant morphological variables (prior to salinity stress) in 19 populations of Spartina patens. PCA was conducted on residuals with a mean of 0 and a variance of 1.0 after removing the effect of block. Variables with high loadings are shown in bold type.

Principal Component	Eigenvalue	Proportion of Variance	Cumulative Proportion of Variance
PC 1	3.495	0.388	0.388
PC 2	2.023	0.225	0.613
PC 3	1.449	0.161	0.774

	Eigenvectors		
Variable	PC 1	PC 2	PC 3
Lethal Salinity Level	0.202	-0.145	**0.557**
Maximum Plant Height	0.258	**0.335**	0.242
Stem Diameter	**0.430**	0.170	-0.298
Internode Distance	0.230	0.008	**-0.547**
Leaf Length	**0.399**	-0.131	**0.391**
Leaf Width	**0.422**	0.279	-0.208
Leaf Length * Width	**0.492**	0.076	0.120
Number of Green Leaves	-0.175	**0.612**	0.135
Total Number of Leaves	-0.213	**0.601**	0.120

Interpretation:
PC 1 Leaf size component with additional positive loading of stem diameter.
PC 2 Leaf number component with additional positive loading of plant height.
PC 3 Salinity component associated with long leaves and relatively short internodes between leaves.

internode component explained an additional 16% of the variation among populations (Table 2.5).

A principal components analysis was also conducted on the same eight morphological variables as above, but without lethal salinity level. Interpretation of the first two principal components, which explained 67% of the variation, was identical to that presented in Table 2.5 with PC1 being a leaf size component and PC2 being a leaf number component. The third component explained an additional 15% of the variation and was interpreted as a short leaf/long internode component. These solely morphological principal component scores were then correlated with lethal salinity level. Lethal salinity level had a highly significant ($P<0.01$) positive correlation with PC1, but the coefficient of correlation was still low ($r=0.27$, $r^2=0.07$). Principal component 2 was not significantly correlated with lethal salinity level. Principal component 3 had a highly significant negative correlation with lethal salinity, but still a moderately low coefficient of correlation ($r=-0.34$, $r^2=0.11$).

DISCUSSION

The results from this study yielded two major findings. First, highly significant intraspecific variation in lethal salinity level was identified among Gulf Coast populations of Spartina patens. Second, populations also displayed significant intraspecific variation in morphological characteristics prior to salinity stress, and in leaf rolling and growth responses during the salinity excursion. However, a strong association between salt tolerance and morphological traits or growth responses was not apparent.

Results from this study generally support and expand upon earlier work that has suggested that variation in salt tolerance exists in subpopulations of Spartina patens. Silander (1979) reported adaptive genetic divergence based on root elongation in a saline solution after plants from dune, swale, and marsh subpopulations were de-acclimated from the different field salinities. Although Pezeshki and DeLaune (1991) reported that Spartina patens collected from a brackish-saltwater population grew better at elevated

salinities than Spartina patens collected from a fresh-water-brackish population, it is unclear from their study whether sufficient time was allowed for the plants to de-acclimate from their respective field salinities. As a result, the reported differences between the two populations may have been due, at least in part, to pre-existing acclimation, rather than adaptive genetic divergence. We have shown that there is, in fact, highly significant intraspecific variation in lethal salinity level in Spartina patens populations following three generations of vegetative regrowth and de-acclimation under non-saline conditions.

Furthermore, our data indicate that one cannot assume that populations collected from relatively higher field salinities will necessarily have higher salinity tolerances than populations collected from lower salinity environments, as has been suggested from preliminary data on a small number of populations (Pezeshki and DeLaune 1991; 1995). Based on descriptions and vegetation associations at the field collection sites, which should provide an index of long-term salinity regime, we can make the following observations. The six populations with the highest lethal salinity levels (O, S, E, F, J, and C) were collected over a range of field salinities as follows: a brackish marsh in Florida (USSCS collection), an interior brackish marsh in Texas, a brackish/saline marsh in Texas, a brackish marsh in Texas, a brackish/saline marsh in Texas, and an intermediate marsh in Texas. The ten populations with similar median lethal salinity levels were all collected from brackish marshes in Louisiana and Texas. Most interestingly, the three populations with the lowest lethal salinity levels (A, K, and B) were collected from areas indicative of relatively high salinities, which were a high marsh bordering a barrier island salt pan with halophytic vegetation in Louisiana, a brackish/saline marsh in Louisiana, and a brackish/saline marsh in Texas, respectively. Therefore, field collection sites of relatively higher salinities may not necessarily yield clones that are more salt tolerant once they are de-acclimated from the field. This is in agreement with Silander and Antonovics (1979) who reported that Spartina patens

collected from the margins of salt flats did not display greater salt tolerance than those sampled from dune, swale and marsh transects along the Outer Banks of North Carolina.

Principal components analysis was useful to illustrate intraspecific variation expressed as a combined trait of salt tolerance and plant production under increasing salinity stress. When populations were ranked in terms of lethal salinity level and also ranked in terms of covariable-adjusted total plant dry weight two different rankings were obtained (Figure 2.1). A principal components analysis of these two variables yielded a first principal component that had high positive loadings of both desirable traits and was able to explain 74% of the variation among populations in these two variables. Analysis of variance of the principal component scores confirmed intraspecific variation in the combined trait of salt tolerance and plant production under salt stress. Therefore, populations characterized by high salt tolerance and also high production under salt stress are identified as those populations with high principal component 1 scores (Figure 2.1). Although there is no guarantee that a principal components analysis will yield a first component that has positive loadings of all the desirable traits entered into the analysis, this technique may have promise where selection for more than one trait is desirable, such as selection of superior planting stocks for use in coastal marsh creation/restoration projects.

Population level leaf rolling response at 30‰ was significantly correlated with ultimate lethal salinity level. At a salinity of 30‰, those populations that displayed less leaf rolling had higher lethal salinity levels than populations exhibiting more tightly rolled leaves (r^2=0.29). Although linear relationships between leaf rolling and water potential, or the degree of water stress, have been reported (O'Toole and Cruz 1980; Begg 1980), we found leaf rolling in S. patens to respond quite variably among populations at salinities less than 30‰, and not in a manner correlated with lethal salinity level (Figure 2.2). Once salinities exceeded 30‰ and leaf elongation rates were already severely depressed, a general trend of more tightly rolled leaves with increasing salinities was

observed (Figure 2.2). Heckathorn and DeLucia (1991) reported that in Spartina pectinata leaf rolling was of secondary importance to stomatal closure as a means of decreasing water loss. O'Toole and Moya (1978) reported that although leaf rolling in rice may be a useful means of screening genotypes for drought tolerance, caution must be exercised when screening widely divergent genotypes since variation in the relationship of water potential and leaf rolling may occur.

Leaf expansion rate appeared more sensitive to sublethal salinity stress than leaf rolling. All populations showed a decrease in leaf expansion rate as salinity increased. Leaf expansion is known to be sensitive to cell turgor pressure, and hence, the water status of the plant (Hsaio 1973; Turner and Begg 1981 and references therein). Although leaf expansion rate at 20‰ was able to explain 21% of the variation in covariable-adjusted total plant dry weight, it only explained 6% of the variation in lethal salinity level among individual experimental units and did not yield a significant correlation with lethal salinity at the population level.

Results from the principal components analysis of plant morphological variables revealed that the Spartina patens populations investigated in this study were readily differentiated on the basis of plant morphological variables. The first two principal components were able to explain 67% of the variation among populations with high loadings of variables associated with leaf size and number, plant height and stem diameter. When lethal salinity level was entered into the analysis, it contributed little to the first two principal components, but had a high positive loading on the third principal component, which explained an additional 16% of the variation among populations (Table 2.5). This salinity component was associated with a negative loading of internode distance and a positive loading of leaf length. Results from the correlation of principal component scores (based only on morphological variables) with lethal salinity support a similar conclusion of long leaves and short internodes being correlated with salt tolerance. Correlation analyses of single variables also showed that leaf length had the

highest correlation with lethal salinity, but that the correlation was weak at the level of the individual experimental unit (r^2=0.18), and still relatively weak at the population level (r^2=0.35; Table 2.3). Therefore, although leaf length is consistently correlated, or associated, with salt tolerance in the populations investigated in this study, salt tolerance cannot be reliably inferred from this trait, or from the other morphological traits and growth responses measured in this investigation.

This study has demonstrated that Gulf Coast populations of Spartina patens display significant intraspecific variation in salinity tolerance. Intraspecific variation was also observed in morphological traits, leaf rolling and growth responses, but was generally not strongly associated with the observed population differentiation in salt tolerance, suggesting that other factors, such as physiological or biochemical differences between populations, are involved in differential salt tolerance. Our future research will focus on investigating differences in physiological and biochemical responses in a subset of these populations, ranging from highly salt tolerant to poorly salt tolerant, when they are subjected to sublethal salinity levels.

Chapter 3

Intraspecific Variation in Salt Tolerance and Morphology in Panicum hemitomon and Spartina alterniflora

INTRODUCTION

Issues of wetland loss and wetland preservation are of concern both nationally and globally. One of the potential causes of stress and ultimate death of wetland vegetation is increased salinity, which may result from canalization, storm surges associated with severe storm events and hurricanes, and sea level rise (Turner et al. 1982; Mendelssohn et al. 1983; Salinas et al. 1986). Although it is well known that wetland plant species exhibit considerable interspecific variation in salt tolerance across coastal plant communities, very little is known concerning the amount of variation in salt tolerance within a species. There are no published studies to date on intraspecific variation in salt tolerance in Panicum hemitomon, and only preliminary findings based on two populations of Spartina alterniflora, which indicate that population differentiation to salinity may occur in this species (Pezeshki and DeLaune 1995). If significant intraspecific variation in salt tolerance exists across populations of these two dominant wetland plant species, the ability to screen these populations for superior salt tolerance may provide a means of identifying salt-tolerant genotypes best suited for marsh restoration and marsh creation projects, as well as identify genotypes for future research on the underlying physiological and biochemical mechanisms associated with superior salt tolerance.

Panicum hemitomon Schult. is distributed continuously along the coastal plain of the United States from New jersey southward into Florida and westward along the Gulf coast into Texas. It is also found in some fresh marshes in Tennessee, as well as in South America (Godfrey and Wooten 1979). Throughout its range Panicum hemitomon

is a common species in the fresh marsh plant community and in southeastern Louisiana it is the dominant emergent macrophyte of the coastal fresh marshes (Chabreck 1972).

Spartina alterniflora Loisel. is a very important coastal salt marsh species that is distributed continuously along the Atlantic coast from New Foundland southward to Florida and westward along the Gulf coast to Texas (Godfrey and Wooten 1979). Spartina alterniflora is generally the dominant coastal salt marsh grass throughout its range, and in Louisiana it dominates the vast southern expanses of the State's coastal salt marshes (Chabreck 1972).

The objective of this study was to determine whether geographically distinct stands (hereafter referred to as populations) of Panicum hemitomon and Spartina alterniflora, dominant emergent macrophytes of fresh marsh and salt marsh plant communities, respectively, display significant intraspecific variation in lethal salt tolerance. A secondary objective was to determine whether intraspecific variation in morphological characteristics, leaf expansion, and leaf rolling could also be identified. If significant intraspecific variation was detected in salt tolerance, plant morphology and growth response, a final objective was to investigate whether a correlation exists between salt tolerance and these other plant traits and responses, such that these characteristics or responses may be used to aid in facilitating future screenings for superior salt tolerance.

In this manuscript we demonstrate that intraspecific variation in salt tolerance and morphology does exist among populations of both of these important coastal grass species and discuss the potential basis for the observed differences between species in the relative importance of morphology in explaining salt tolerance.

MATERIALS AND METHODS

Plant material

Clones from 19 geographically distinct populations of Panicum hemitomon were collected from Louisiana coastal fresh marshes by the US Soil Conservation Service Plant Materials Laboratory. Field collection sites for the 25 Spartina alterniflora clones

covered a broader geographical area that extended from eastern Louisiana to south Texas and included brackish/saline marshes, salt marshes, and irregularly flooded salt marshes that bordered salt pans. Single stems from each population were vegetatively propagated over the course of four to six generations under uniform, non-saline conditions in the greenhouse to reduce any field acclimation to varying salinity conditions and to ensure that each population was represented by a single genotype. Propagation was accomplished by physically separating new tillers (ramets) from the parent ramet, discarding the parent and then repotting the young ramets. This process was repeated four times for Spartina alterniflora and six times for Panicum hemitomon. Plants were propagated in a commercial potting mix (Jiffy Mix®; Chicago Illinois) and all pots were given equal amounts of Hoagland's nutrient solution (Hoagland and Arnon 1950) as needed to produce vigorous growth.

Experimental design

The salinity screening experiments were set up as two separate randomized block designs of five replicates each. The Spartina alterniflora salinity screening experiment was conducted in a glasshouse on the LSU, Baton Rouge, Louisiana campus during the early summer through fall. Maximum daytime temperatures averaged approximately 36 C during mid summer and approximately 30 C during the fall, with the corresponding illumination (photon flux density) inside the glasshouse ranging from approximately 1000 to 1200 μmoles m^{-2} s^{-1}. The Panicum hemitomon salinity screening experiment was conducted in a temperature-controlled EGC walk-in growth chamber set to 16 hr daylength at 30 C and an 8 hr dark period at 24 C. Illumination (photon flux density) inside the growth chamber was approximately 1300 - 1400 μmoles m^{-2} s^{-1} at canopy height. Depending on the size of the plants, two to four young stems were planted per pot to minimize weight differences in the amount of initial plant material per experimental unit. Initial plant wet weight was also recorded and tested for statistical significance as a covariable for harvest biomass. Five replicates of each of the populations were potted in

Jiffy Mix in 0.7 liter plastic pots equipped with bottom and side drainage holes. The pots were placed inside larger reservoir pots that contained the treatment bathing solutions of increasing salinity through time. Treatment bathing solutions were maintained at a level of 4 to 5 cm below the soil surface. This allowed exchange of soil interstitial water with the treatment bathing solution without flooding the soil surface.

Salinity regime

After transplanting, all experimental units were maintained in a treatment bathing solution of half-strength (50%) Hoagland's nutrient solution at 0‰ salinity for 20 days. Salinity was then increased stepwise in weekly increments in half-strength Hoagland's using a commercial synthetic sea salts mix (Instant Ocean®; Aquarium Systems, Mentor, Ohio) with major ionic composition expressed as percentage of dry weight as follows: 46.9% Cl, 26.0% Na, 6.4% SO_4, 3.2% Mg, 1.0% Ca, and 0.9% K (Bidwell and Spotte 1985). Panicum hemitomon was subjected to weekly salinity increases of 2‰, whereas Spartina alterniflora was subjected to weekly increases of 10‰. All salinity increases throughout both experiments followed the following procedure: 1) Pots were removed from reservoir containers and allowed to drain for one hour. 2) Pots were then flushed twice with 350 ml of Instant Ocean solution at a salinity that was 50% higher than the desired step increase by slowly adding the solution to the soil surface and allowing it to drain through the soil and out the drainage holes over a period of two hours. Therefore, Panicum hemitomon flushing was done with a solution that was 1‰ higher than the desired targeted salinity and Spartina alterniflora flushing was done with a solution that was 5‰ higher than the desired targeted salinity. 3) Pots were placed back inside the reservoir containers. 4) A solution of half-strength strength Hoagland's with Instant Ocean at the targeted salinity was added to the soil surface in two 350 ml aliquots, which drained through the soil and collected in the reservoir containers, bringing the solution in the reservoir up to the proper level. Salinity was rechecked for accuracy. Solution levels and salinities were monitored daily.

Variables measured

The following plant morphological variables were measured on a randomly selected stem from a pool of representative stems in each pot immediately prior to salinity increase: plant height (to the uppermost leaf ligule, i.e. to the ligule of the youngest expanded leaf; to the tip of the emerging terminal leaf; and maximum height to the tallest vertically straightened leaf tip), leaf length and width (on the top two expanded leaves below the terminal leaf, hereafter referred to as leaf one and leaf two), internode distance between leaves one and two and between leaves two and three, stem diameter measured between leaves one and two and between leaves two and three, and total number of expanded leaves per stem.

Leaf expansion was measured on two randomly selected stems from a pool of stems having terminal leaves that were neither newly emergent nor fully expanded, but in the range of one third to two thirds expanded. For Spartina alterniflora leaf length was measured from the tip of the terminal leaf to the ligule of the youngest expanded leaf over a time interval of three days. For Panicum hemitomon measurements of leaf expansion were taken from the tip of the terminal leaf to a fixed point (the rim of the pot) over a time interval of three days, and thus represent a composite of expansion of the terminal leaf in addition to any stem elongation below the ligule of the first expanded leaf that occurred during that time interval. These methods have been used successfully with other grasses and produced results which parallel changes in aboveground biomass and photosynthesis (Hester and Mendelssohn 1990; Koch et al. 1990). Panicum hemitomon leaf expansion measurements were made at salinities of 0‰, 2‰, 4‰, and 6‰. Spartina alterniflora leaf expansion measurements were made at salinities of 2‰, 15‰, 35‰, 55‰, and 75‰.

Other studies have shown that leaf rolling may be an indicator of plant water stress that can be utilized in screening crop plants for drought tolerance and , hence, may have value in screening for salt tolerance (O'Toole and Moya 1978; O'Toole and Cruz

1980). Leaf rolling was measured on the top two expanded leaves of the stems tagged for leaf expansion measurements. A leaf rolling index was calculated at the midpoint of the leaf as the projected width of the leaf divided by the unrolled width of the leaf (Begg 1980). Therefore, a leaf that is not rolled will have an index of 1.0, whereas leaves that are rolled will have indices less than 1.0. Leaf rolling measurements were taken at the sublethal salinity level of 4‰ for Panicum hemitomon and 35‰ for Spartina alterniflora.

Plants were harvested when there was 50% death of aboveground tissue (comparable to an LD_{50}) as determined by visual assessment of the relative amounts of live (green) and dead (brown) aboveground tissue. Once a greater proportion of brown tissue was observed, the experimental unit was harvested. Aboveground tissue was lightly rinsed with tap water to remove any encrusted salts on the leaf surfaces. Soil was rinsed from the roots, and the plant was partitioned into aboveground and belowground components. After blotting the tissue to remove excess moisture, aboveground and belowground wet weights were determined. Samples were oven dried at 65 C for five days until constant weight was achieved and dry weight determined.

Data analysis

Data was analyzed as a randomized block design. Analysis of variance (ANOVA) was used to test for significant population differences using SAS (SAS 1989; Steel and Torrie 1980). A significance level (alpha) of 0.05 was used for all statistical analyses unless otherwise stated. All data were tested for meeting the assumptions of normality (Shapiro-Wilk test statistic) and homogeneity of variance (Bartlett test statistic) using SAS (SAS 1989). Panicum hemitomon leaf rolling index was arcsin transformed and number of leaves per stem was square-root transformed to meet the assumptions of normality and homogeneity of variance. For Spartina alterniflora leaf rolling index was similarly arcsin transformed, stem number, total plant dry weight and covariable-adjusted total plant dry weight were log transformed, and leaf length, plant height to the upper

ligule (ligule of the first expanded leaf) and internode distance were square-root transformed to meet these assumptions.

Correlation matrices were constructed to detect potentially useful linear relationships between plant morphological variables, leaf elongation rates, and leaf rolling indices with lethal salinity level, and covariable-adjusted total plant dry weight. Correlations were conducted at two levels: 1) at the level of the individual based on all experimental units (n=95 for Panicum hemitomon; n=125 for Spartina alterniflora) and 2) at the population level based on population means (n=19 for Panicum hemitomon; n=25 for Spartina alterniflora). Correlations conducted at the population level are justified because it is at the level of the population that selection for superior salt tolerance was made. Gomez and Gomez (1984) further recommend the use of treatment (or population) mean correlations to avoid confounding the residual error with variation between experimental units of the same population (i.e., experimental error).

Principal components analysis (PCA; SAS 1989) was used 1) to investigate intraspecific variation expressed in a created variable based on positive loadings of two desirable salinity tolerance traits: plant production under increasing salinity stress and lethal salinity level, 2) to investigate relationships in the principal component loadings of lethal salinity level and morphological traits, and 3) to obtain principal component scores based solely on plant morphological data that were then correlated with salt tolerance (Johnson and Wichern 1988).

RESULTS

Salt tolerance

Highly significant ($P<0.01$) differences were found among Panicum hemitomon populations in lethal salinity level (salinity that resulted in 50% death of aboveground tissue). Lethal salinity levels ranged from a low tolerance of 7.6‰ to a high tolerance of 12.0‰, with several of the populations having lethal salinity levels near 10‰ (Figure 3.1 top panel).

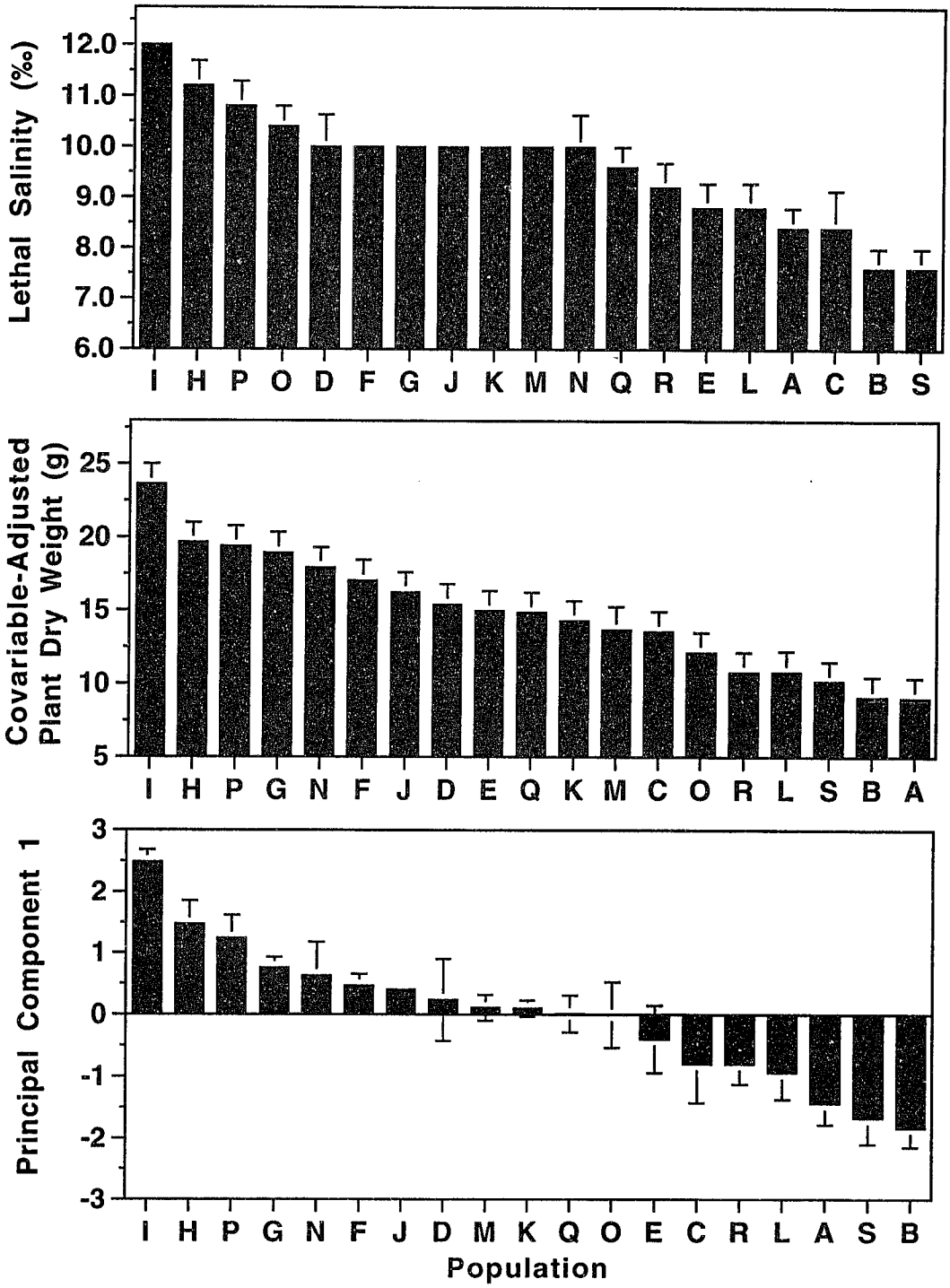

Figure 3.1. Lethal salinity level (salinity resulting in 50% death of aboveground tissue; top panel; $LSD_{0.05}$=1.15; MSE=0.83), covariable-adjusted total plant dry weight (middle panel, $LSD_{0.05}$=3.88, MSE=9.47), and principal component one scores (bottom panel, $LSD_{0.05}$=1.11, MSE=0.78) from a principal components analysis of lethal salinity level and covariable-adjusted total plant dry weight for 19 populations of Panicum hemitomon (n=5).

Populations of Spartina alterniflora also displayed highly significant intraspecific variation in lethal salinity level. Maximum salinity tolerance was 115‰ for five of the populations followed by a steady decrease down to 93‰ - 95‰, with one population displaying the lowest tolerance at 83‰ (Figure 3.2 top panel).

Panicum hemitomon displayed highly significant ($P<0.01$) population differences in wet and dry aboveground, belowground, total, and covariable-adjusted total plant biomass (Table 3.1). Wet weight data followed the same trends as dry weight data and are not presented. Aboveground dry weight ranged from 6.1 to 17.5 g per pot (Table 3.1). Biomass rankings based on covariable-adjusted total plant dry weight are presented in Figure 3.1 (middle panel) and show a range from 9.0 to 23.6 g per pot. Belowground-to-aboveground dry weight ratios also displayed highly significant differences among populations and ranged from 0.23 to 0.48 (Table 3.1).

Populations of Spartina alterniflora also displayed highly significant intraspecific variation in all plant biomass parameters (Table 3.1). Aboveground dry weight ranged from 4.1 to 10.4 g per pot (Table 3.1). Covariable-adjusted total plant dry weight ranged from 7.5 to 16.7 g per pot (Table 3.1; Figure 3.2 middle panel). Belowground-to-aboveground dry weight ratios ranged from 0.32 to 0.96 (Table 3.1).

For both species, the covariable, initial plant wet weight at the time of planting, showed significant differences between populations despite efforts to minimize this effect. Therefore, only covariable-adjusted total plant dry weight is used throughout the remainder of this manuscript.

A principal components analysis of Panicum hemitomon lethal salinity level and covariable-adjusted plant dry weight was conducted to investigate intraspecific variation expressed as a derived variable that combined these two desirable traits. Figure 3.1 shows the relationship between lethal salinity level, covariable-adjusted plant dry weight and the first principal component, which had high positive loadings of both traits and was able to explain 90% of the variation among Panicum hemitomon populations in these

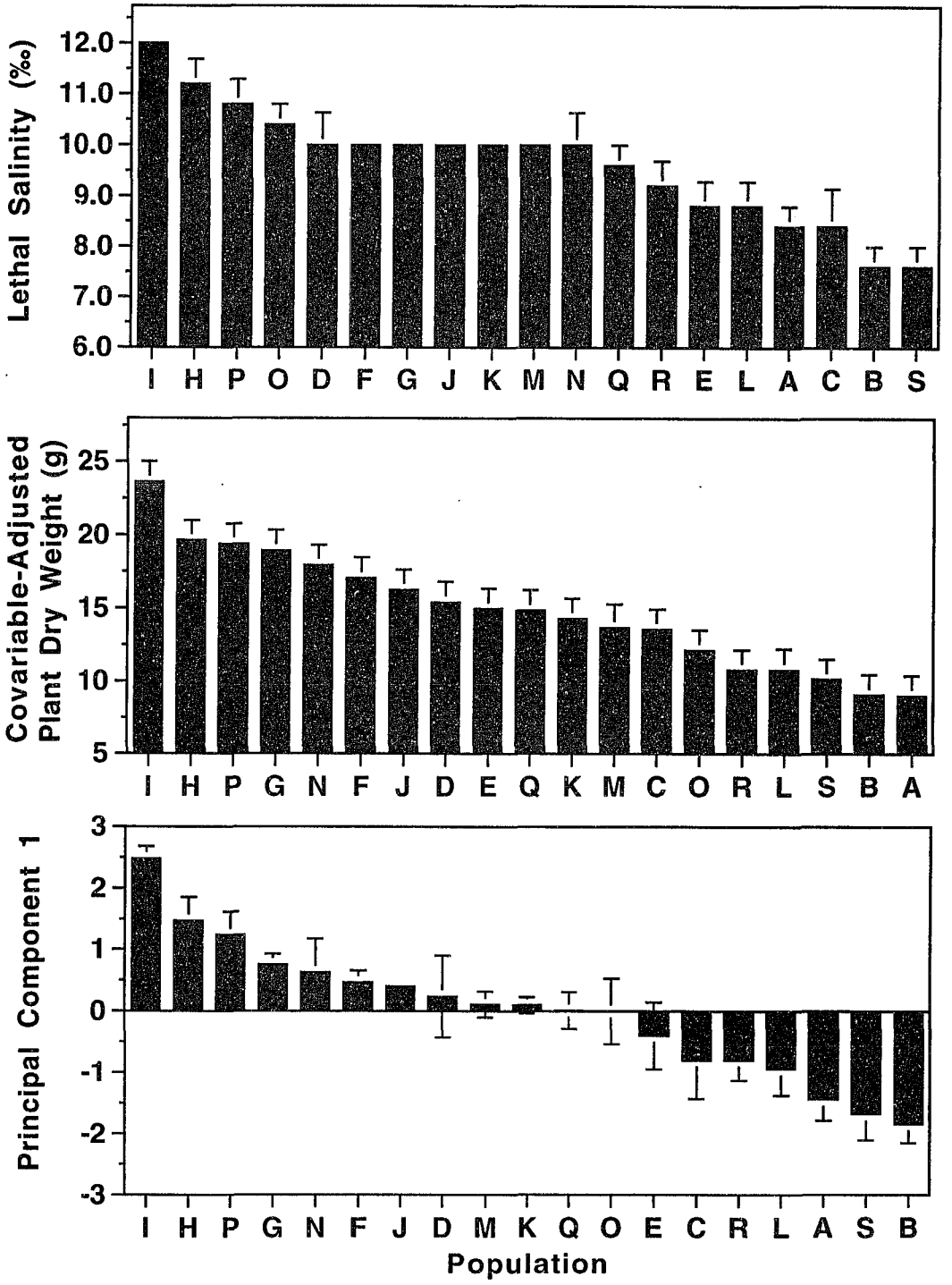

Figure 3.2. Lethal salinity level (salinity resulting in 50% death of aboveground tissue; top panel; $LSD_{0.05}$=7.70; MSE=37.62), covariable-adjusted total plant dry weight (middle panel, $LSD_{0.05}$=3.10, MSE=6.11), and principal component one scores (bottom panel, $LSD_{0.05}$=0.97, MSE=0.60) from a principal components analysis of lethal salinity level and covariable-adjusted total plant dry weight for 25 populations of Spartina alterniflora (n=5).

Table 3.1. Range of population mean leaf rolling indices and plant biomass parameters in 19 populations of Panicum hemitomon (top panel) and Spartina alterniflora (bottom panel) harvested at their respective lethal salinity levels, the resultant F values and probability levels of a significant population difference (n=5). Biomass is expressed as g dry weight per pot.

Panicum hemitomon

Variable	Range Minimum	Range Maximum	F value (18, 72 df)	Prob > F
Leaf Rolling Index	0.74	0.99	3.86	.0001
Aboveground Biomass	6.1	17.5	6.73	.0001
Belowground Biomass	2.4	7.1	8.72	.0001
Total Biomass	8.9	24.6	7.47	.0001
Total Covariable-Adjusted Biomass	9.0	23.6	8.42	.0001
Belowground to Aboveground Ratio	0.23	0.48	6.54	.0001

Spartina alterniflora

Variable	Range Minimum	Range Maximum	F value (24, 96 df)	Prob > F
Leaf Rolling Index	0.76	1.00	6.60	.0001
Aboveground Biomass	4.1	10.4	3.59	.0001
Belowground Biomass	1.7	6.5	3.60	.0001
Total Biomass	6.1	16.9	3.36	.0001
Total Covariable-Adjusted Biomass	7.5	16.7	3.94	.0001
Belowground to Aboveground Ratio	0.32	0.96	5.89	.0001

two variables. An ANOVA of the principal component 1 scores revealed highly significant population differences. Based on this analysis, populations with high principal component 1 scores are classified as highly salt tolerant, characterized by high production under salinity stress and also high lethal salinity level. Populations with low scores are classified as poorly salt tolerant, characterized by low production under salinity stress and low lethal salinity level.

The principal components analysis of lethal salinity level and covariable-adjusted plant dry weight for Spartina alterniflora did not explain as much variation in the first principal component as was found in Panicum hemitomon. Figure 3.2 shows the relationship between lethal salinity level, covariable-adjusted plant dry weight and the first principal component, which had high positive loadings of both traits and explained 66% of the variation among populations for Spartina alterniflora compared to 90% for Panicum hemitomon. An ANOVA of the principal component 1 scores, however, did reveal highly significant population differences, thereby allowing population identification as described above for Panicum.

Panicum hemitomon populations showed a trend of decreasing leaf expansion rate with increasing salinity level. Figure 3.3 (top panel) shows leaf expansion rates on a subset of six Panicum hemitomon populations ranging from highly salt tolerant to poorly salt tolerant. Significant population differences in leaf expansion rate were detected at 0‰, 2‰ and 4‰. At 6‰ there were no longer significant population differences.

Significant populations differences in leaf expansion rates of Spartina alterniflora populations were detected at all five of the sublethal salinity levels measured. As was found for Panicum, Spartina alterniflora populations showed a trend of decreasing leaf expansion rate with increasing salinity level and populations did not maintain a consistent ranking of leaf expansion rates across increasing salinities (Figure 3.3 bottom panel)

There were highly significant ($P<0.01$) differences among Panicum hemitomon populations in the extent to which leaves were rolled at 4‰ (Table 3.1). Leaf rolling

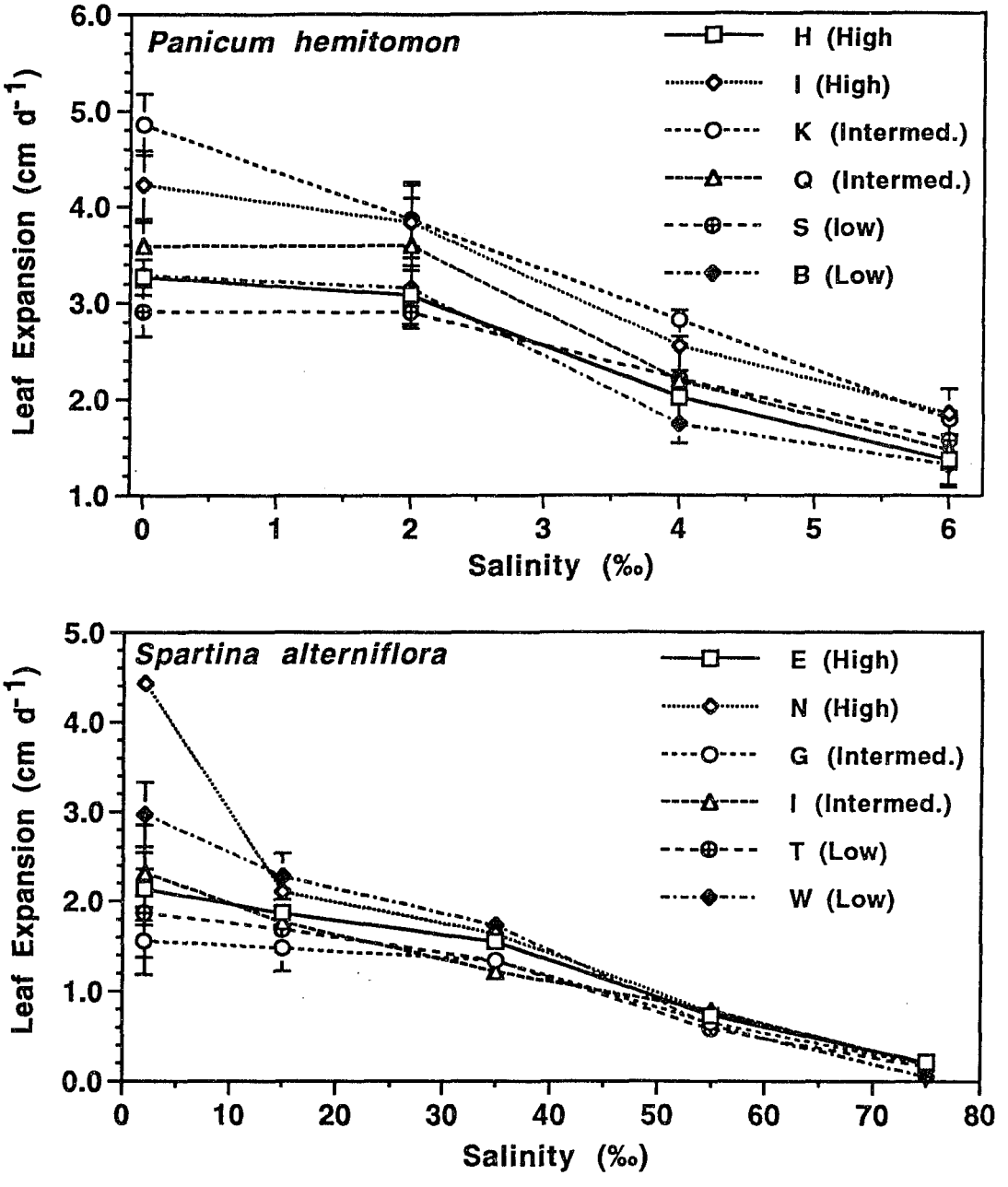

Figure 3.3. Leaf expansion rates for subsets of 6 of the 19 populations of Panicum hemitomon (top panel) and 6 of the 25 populations of Spartina alterniflora (bottom panel) as salinity was increased in weekly increments of 2‰ for Panicum hemitomon and 10‰ for Spartina alterniflora. Shown are two populations for each species with high lethal salinity levels (high), two populations with intermediate lethal salinity levels (intermed.), and two populations with low lethal salinity levels (low), n=5.

indices ranged from 0.99 (essentially unrolled) to 0.74 (projected leaf width reduced by 26% through rolling; Table 3.1).

Spartina alterniflora populations also displayed highly significant differences in the degree to which leaves were rolled at a sublethal salinity level. At 35‰ leaf rolling index ranged from 1.00 (leaves not rolled) to 0.76 (projected leaf width reduced by 24% through rolling; Table 3.1).

Plant morphology

With the exception of number of leaves per stem and internode distance between leaves one and two, all other plant morphological variables measured prior to salinity stress displayed highly significant ($P<0.01$) differences among Panicum hemitomon populations (Table 3.2). Leaf widths ranged from 1.21 to 1.69 cm with leaf lengths ranging from 17.7 to 30.4 cm (Table 3.2).

Highly significant ($P<0.01$) differences among Spartina alterniflora populations were found in all the plant morphological variables measured prior to salinity stress (Table 3.3). Unlike Panicum, Spartina alterniflora populations did display significant intraspecific variation in internode distance between leaves one and two, which ranged from 0.38 to 7.96 cm (Table 3.3). The number of leaves per stem ranged from 4.8 to 8.8 (Table 3.3). Leaf widths ranged from 0.45 to 0.87 cm and leaf lengths ranged from 31.1 to 60.3 cm (Table 3.3).

Correlations with salt tolerance and plant production

Panicum hemitomon leaf expansion rates at 0‰ and 4‰ were significantly correlated with lethal salinity level (Table 3.4). However, the strength of these correlations were fairly weak with the respective r^2 values explaining less than 7% of the variation in lethal salinity level among individual experimental units and were not significant when based on population means (Table 3.4).

Table 3.2. Range of population means (n=5) for plant morphological variables in 19 populations of Panicum hemitomon following three generations of growth under non-saline conditions, the resultant F values (18, 72 df), and probability levels of a significant population difference.

Variable	Range Minimum	Range Maximum	F value	Probability > F
Plant Height (cm)				
to upper ligule	24.10	34.90	2.72	0.0014
to growing tip	36.64	54.70	2.54	0.0028
to tallest leaf tip	45.84	65.32	3.57	0.0001
Leaves per Stem	3.2	5.0	1.31	0.2095
Internode Distance (cm)				
between leaves 1-2	2.55	4.90	0.59	0.8963
between leaves 2-3	5.14	9.19	1.82	0.0385
Stem Diameter (cm)				
between leaves 1-2	0.22	0.32	2.39	0.0048
between leaves 2-3	0.28	0.41	2.10	0.0144
Leaf				
Length (cm)[a]	17.73	30.40	3.19	0.0002
Width (cm)[a]	1.21	1.69	4.35	0.0001
Length x Width (cm^2)[a]	23.67	47.27	3.62	0.0001

[a]measured on the top two expanded leaves.

Table 3.3. Range of population means (n=5) for plant morphological variables in 25 populations of Spartina alterniflora following three generations of growth under non-saline conditions, the resultant F values (24, 96 df), and probability levels of a significant population difference.

Variable	Range Minimum	Maximum	F value	Probability > F
Plant Height (cm)				
to upper ligule	8.32	43.04	6.56	0.0001
to growing tip	18.94	62.22	9.97	0.0001
to tallest leaf tip	51.24	87.60	5.21	0.0001
Leaves per Stem	4.8	8.8	3.65	0.0001
Internode Distance (cm)				
between leaves 1-2	0.38	7.96	6.30	0.0001
between leaves 2-3	0.50	7.42	5.79	0.0001
Stem Diameter (cm)				
between leaves 1-2	0.19	0.47	4.56	0.0001
between leaves 2-3	0.25	0.53	6.50	0.0001
Leaf				
Length (cm)[a]	31.11	60.25	7.26	0.0001
Width (cm)[a]	0.45	0.87	5.89	0.0001
Length x Width (cm^2)[a]	14.71	49.79	7.37	0.0001

[a]measured on the top two expanded leaves.

Table 3.4. Pearson correlation coefficients (r) and significance levels (prob > | r |) for correlations of Panicum hemitomon (Top panel; n=95) and Spartina alterniflora (bottom panel; n=125) leaf expansion rates and leaf rolling index at the indicated salinity levels with lethal salinity level and covariable-adjusted total plant dry weight. Significant (P<0.05) correlations are shown in bold face type. Significant correlations based on population means (n=19 for Panicum; n=25 for Spartina) are shown in bold face type within parantheses as a second row for the corresponding variable pairs.

Panicum hemitomon

	Lethal Salinity Level		Covariable-adjusted Total Plant Dry Weight	
	r	prob > | r |	r	prob > | r |
Leaf expansion				
0‰	**0.257**	**0.012**	**0.094**	**0.004**
2‰	0.112	0.280	0.091	0.063
4‰	**0.253**	**0.014**	0.195	0.059
6‰	0.107	0.301	0.188	0.069
Leaf rolling index				
4‰	0.001	0.990	-0.047	0.648

Spartina alterniflora

	Lethal Salinity Level		Covariable-adjusted Total Plant Dry Weight	
	r	prob > | r |	r	prob > | r |
Leaf expansion				
2‰	0.017	0.850	**0.392**	**0.0001**
15‰	-0.068	0.452	**0.430** **(0.406)**	**0.0001** **(0.044)**
35‰	0.069	0.444	**0.371**	**0.0001**
55‰	**0.305** **(0.404)**	**0.001** **(0.045)**	**0.213**	**0.017**
75‰	**0.407** **(0.547)**	**0.0001** **(0.005)**	0.134	0.137
Leaf rolling index				
35‰	**0.402** **(0.617)**	**0.0001** **(0.001)**	0.051	0.569

Leaf expansion rates of individual experimental units of Panicum hemitomon at 0‰ were significantly correlated with covariable-adjusted total plant dry weight, but explained less than 9% of the variation in total plant dry weight (Table 3.4).

Leaf expansion rates of Spartina alterniflora at 55‰ and 75‰ were significantly correlated with lethal salinity level (Table 3.4). The correlation at 75‰ was the stronger of the two, but only explained 17% of the variation in lethal salinity level (Table 3.4). At the population level leaf expansion at 55‰ and 75‰ were also correlated with lethal salinity level and were able to explain 16% and 17%, respectively, of the variation in lethal salinity level among populations (Table 3.4).

Leaf expansion rates of Spartina alterniflora populations at 2‰, 15‰, 35‰ and 55‰ were significantly correlated with covariable-adjusted total plant dry weight (Table 3.4). Of these, the strongest correlation was leaf expansion at 15‰, which explained 18% of the variation in covariable-adjusted total plant dry weight (Table 3.4). At the population level leaf expansion at 15‰ was significantly correlated with covariable-adjusted total plant dry weight, explaining 16% of the variation in lethal salinity level among populations (Table 3.4).

Leaf rolling index of Panicum hemitomon at 4‰ failed to significantly correlate with lethal salinity level or covariable-adjusted total plant dry weight at the level of the individual experimental unit or at the population level (Table 3.4)

Unlike Panicum hemitomon, leaf rolling of Spartina alterniflora measured at the sublethal salinity level of 35‰ displayed a significant positive correlation with lethal salinity level, indicating that leaves were less rolled in the more salt-tolerant populations (Table 3.4). This leaf rolling correlation explained 16% of the variation in lethal salinity tolerance at the level of the individual experimental unit and 38% of the variation in lethal salinity tolerance among populations (Table 3.4).

Many of the Panicum hemitomon plant morphological variables measured prior to salinity increase had significant correlations with lethal salinity level and

covariable-adjusted total plant dry weight (Table 3.5). Lethal salinity level of individual experimental units had significant positive correlations with plant height (measured to the tallest leaf tip), stem diameter between leaves one and two and between leaves two and three, leaf length, leaf width and leaf length x width (Table 3.5). With the exception of internode distances and number of leaves per stem, all plant morphological characteristics were significantly positively correlated with covariable-adjusted total plant dry weight (Table 3.5). Leaf length and leaf length x width had the strongest correlations and were both able to explain 18% of the variation in lethal salinity level and 23% and 20%, respectively, of the variation in covariable-adjusted total plant dry weight among experimental units (Table 3.5).

Panicum hemitomon morphological correlations were stronger at the level of the population. Leaf length and leaf length x width explained 43% and 46% of the variation in lethal salinity level and 43% and 35% of the variation in covariable-adjusted total plant dry weight among populations, respectively (Table 3.5).

No significant morphological correlations with lethal salinity level were detected in Spartina alterniflora at the level of the individual experimental unit or at the population level (Table 3.6). With the exception of plant height to the tallest leaf tip and leaf length, all other plant morphological characteristics had significant correlations with covariable-adjusted total plant dry weight (Table 3.6). Number of leaves per stem explained 15% of the variation in covariable-adjusted total plant dry weight (Table 3.6).

At the population level there were no significant morphological correlations with Spartina alterniflora lethal salinity level. Morphological correlations with covariable-adjusted total plant dry weight at the population level yielded significant positive correlations with plant height to the upper ligule and number of leaves per stem, and a negative correlation with stem diameter measured between leaves two and three (Table 3.6).

Table 3.5. Pearson correlation coefficients (r) and significance levels (prob > | r |) for correlations of Panicum hemitomon plant morphological variables prior to salinity stress and belowground to aboveground (dry weight) ratio at harvest with lethal salinity level and covariable-adjusted total plant dry weight. Significant (P<0.05) correlations are shown in bold face type (n=95). Significant correlations based on population means (n=19) are shown in bold face type within parantheses as a second row for the corresponding variable pairs.

	Lethal Salinity Level		Covariable-adjusted Total Plant Dry Weight	
	r	prob > | r |	r	prob > | r |
Plant Height				
to upper ligule	0.155	0.133	**0.225**	**0.029**
to growing tip	0.186	0.071	**0.241**	**0.019**
to tallest leaf tip	**0.332**	**0.001**	**0.406**	**0.0001**
	(0.466)	**(0.045)**		
Leaves per Stem	0.158	0.126	0.164	0.112
Internode Distance				
between leaves 1-2	0.094	0.364	0.131	0.206
between leaves 2-3	-0.046	0.660	-0.030	0.776
Stem Diameter				
between leaves 1-2	**0.232**	**0.024**	**0.254**	**0.013**
	(0.510)	**(0.026)**	**(0.539)**	**(0.017)**
between leaves 2-3	**0.304**	**0.003**	**0.326**	**0.001**
	(0.543)	**(0.016)**	**(0.523)**	**(0.022)**
Leaf				
Length	**0.430**	**0.0001**	**0.478**	**0.0001**
	(0.660)	**(0.002)**	**(0.655)**	**(0.002)**
Width	**0.288**	**0.005**	**0.239**	**0.020**
Length x Width	**0.425**	**0.0001**	**0.448**	**0.0001**
	(0.680)	**(0.001)**	**(0.590)**	**(0.008)**
Belowground to Aboveground Ratio	**-0.180**	**0.081**	0.069	0.507

Table 3.6. Pearson correlation coefficients (r) and significance levels (prob > | r |) for correlations of Spartina alterniflora plant morphological variables prior to salinity stress and belowground to aboveground (dry weight) ratio at harvest with lethal salinity level and covariable-adjusted total plant dry weight. Significant (P<0.05) correlations are shown in bold face type (n=95). Significant correlations based on population means (n=19) are shown in bold face type within parantheses as a second row for the corresponding variable pairs.

	Lethal Salinity Level		Covariable-adjusted Total Plant Dry Weight	
	r	prob > | r |	r	prob > | r |
Plant Height				
to upper ligule	0.080	0.374	**0.334**	**0.0001**
			(0.438)	**(0.028)**
to growing tip	-0.046	0.613	**0.236**	**0.008**
to tallest leaf tip	-0.050	0.581	0.171	0.056
Leaves per Stem	-0.080	0.373	**0.389**	**0.0001**
			(0.399)	**(0.048)**
Internode Distance				
between leaves 1-2	-0.035	0.697	**0.205**	**0.022**
between leaves 2-3	0.053	0.556	**0.244**	**0.006**
Stem Diameter				
between leaves 1-2	-0.046	0.609	**-0.222**	**0.013**
between leaves 2-3	-0.141	0.116	**-0.276**	**0.002**
			(-0.444)	**(0.026)**
Leaf				
Length	-0.093	0.301	-0.016	0.861
Width	-0.097	0.281	**-0.236**	**0.008**
Length x Width	-0.160	0.075	**-0.157**	**0.080**
Belowground to Aboveground Ratio	0.085	0.345	0.133	0.140

Results from the Panicum hemitomon principal components analysis of lethal salinity level and eight plant morphological variables showed that 68% of the variation among populations was explained by the first two principal components (Table 3.7). The third principal component only explained an additional 10% of the variation and, therefore, will not be further discussed. The first principal component was interpreted to be a leaf size and leaf number component with an additional positive loading of plant height and a weak positive loading of lethal salinity (Table 3.7). The second principal component was interpreted to be an internode distance component with an additional positive loading of stem diameter and a negative loading of number of leaves. This second component explained an additional 23% of the variation among populations (Table 3.7).

Results from the Spartina alterniflora principal components analysis of lethal salinity level and eight plant morphological variables showed that 74% of the variation among populations was explained by the first three principal components (Table 3.8). The first two principal components were interpreted to be a leaf size and stem diameter component, and a plant height and leaf number component, respectively, and were able to explain 62% of the variation among populations (Table 3.8). Lethal salinity level had a high loading on the third principal component and was associated with a positive loading of internode distance. This lethal salinity level/long internode component explained an additional 12% of the variation among populations (Table 3.8).

In order to further assess the extent to which salt tolerance could be correlated with plant morphological variables, a principal components analysis was conducted on the same eight morphological variables as above, but without lethal salinity level. These purely morphological principal component scores were then correlated with lethal salinity level. Interpretation of the first two morphological principal components for Panicum hemitomon, which explained 48% and 25% of the variation, respectively, was identical to that presented in Table 3.7 with PC1 being a leaf size/leaf number/plant height

Table 3.7. Eigenvalues, eigenvectors, and interpretation of the first three principal components from a nine variable principal components analysis of lethal salinity level and plant morphological variables (prior to salinity stress) in 19 populations of Panicum hemitomon. Variables with high loadings are shown in bold type.

Principal Component	Eigenvalue	Proportion of Variance	Cumulative Proportion of Variance
PC 1	4.015	0.446	0.446
PC 2	2.089	0.232	0.678
PC 3	0.939	0.104	0.783

	Eigenvectors		
Variable	PC 1	PC 2	PC 3
Lethal Salinity Level	0.256	0.177	**0.453**
Maximum Plant Height	**0.396**	0.193	-0.298
Stem Diameter	0.087	**0.434**	**0.628**
Internode Distance (leaves 2-3)	-0.118	**0.546**	**-0.383**
Leaf Length	**0.429**	0.069	-0.318
Leaf Width	0.328	0.263	0.150
Leaf Length * Width	**0.460**	0.173	-0.161
Number of Green Leaves	**0.375**	**-0.371**	0.081
Total Number of Leaves	0.335	**-0.452**	0.100

Interpretation:

PC 1 Leaf size and number component associated with positive loading of plant height and weak positive loading of lethal salinity.

PC 2 Internode distance component associated with positive loading of stem diameter and negative loading of number of leaves.

PC 3 Stem diameter component associated with positive loading of salinity and negative loading of internode distance.

Table 3.8. Eigenvalues, eigenvectors, and interpretation of the first three principal components from a nine variable principal components analysis of lethal salinity level and plant morphological variables (prior to salinity stress) in 25 populations of Spartina alterniflora. Variables with high loadings are shown in bold type.

Principal Component	Eigenvalue	Proportion of Variance	Cumulative Proportion of Variance
PC 1	3.543	1.531	0.394
PC 2	2.013	0.224	0.617
PC 3	1.074	0.119	0.737

	Eigenvectors		
Variable	PC 1	PC 2	PC 3
Lethal Salinity Level	-0.083	-0.110	**0.593**
Maximum Plant Height	0.196	**0.554**	0.291
Stem Diameter	**0.427**	-0.168	-0.022
Internode Distance	-0.132	0.328	**0.620**
Leaf Length	**0.458**	0.200	-0.068
Leaf Width	**0.479**	-0.044	0.085
Leaf Length * Width	**0.513**	0.127	-0.022
Number of Green Leaves	-0.217	**0.448**	-0.289
Total Number of Leaves	-0.077	**0.535**	-0.287

Interpretation:

PC 1 Leaf size component associated with positive loading of stem diameter.

PC 2 Plant height component associated with positive loadings of numbers of leaves.

PC 3 Internode distance component associated with positive loading of salinity.

component and PC2 being a stem diameter/long internode/few leaves component. Correlation of these first two principal components with Panicum hemitomon lethal salinity showed that only the first principal component was significantly positively correlated with lethal salinity level and yielded a correlation coefficient of 0.40 (r^2=16% of the lethal salinity variance explained by PC1).

As in Panicum hemitomon, the interpretation of the purely morphological principal components for Spartina alterniflora remained similar to the PCA that included lethal salinity. The first principal component was a leaf size/stem diameter component and explained 44% of the variation. The second component was a plant height/leaf number component and explained an additional 25% of the variation. The third component was an internode distance component and explained an additional 13% of the variation. However, unlike Panicum, correlation analysis with Spartina alterniflora morphological principal components yielded no significant correlations with lethal salinity level.

DISCUSSION

There are no published studies to date that have investigated intraspecific variation, or population differentiation, in Panicum hemitomon in terms of plant morphology or differential population responses to environmental stress. Although there has been research on population differentiation in Spartina alterniflora in regard to a genetic basis for the observed tall and short growth forms (Mooring et al. 1971; Shea et al. 1975), the potential for population differentiation in salinity tolerance has only recently been investigated and was conducted on only two populations (Pezeshki and DeLaune 1995). None of the studies to date have investigated intraspecific variation in salt tolerance in these two important coastal grass species across a large geographical area, nor has an attempt been made to quantify salt tolerance in terms of actual lethal salinity level.

This study has shown that populations of both Panicum hemitomon and Spartina alterniflora display highly significant intraspecific variation in lethal salinity level. This is an important finding both from a theoretical and an applied perspective because it documents population differentiation to an important environmental stress and also indicates that the potential exists for selecting superior salt-tolerant genotypes of these two species for use in marsh creation and restoration projects. Another important result of this research is the identification of differences between these two species in the relative importance of plant morphological variables as indicators of salt tolerance. In Panicum hemitomon morphological variables associated with plant and leaf size were highly correlated with lethal salinity level and may prove to be useful for future screenings of salt tolerance, whereas morphological differences among Spartina alterniflora populations appear to be of no value in explaining salt tolerance.

We suggest that in Panicum hemitomon, a fresh marsh dominant, the lack of documented anatomical adaptations for salt tolerance and an apparently limited physiological response to salinity result in plant and leaf size being important in salt tolerance. The mechanism may be a dilution effect where larger size simply results in more tissue being available for salt translocation and storage (Munns 1993). The translocation of ions to older leaves that may then senesce, or be shed, is a documented mechanism of reducing, or removing, toxic ions from the actively growing regions of the plant (Albert 1975; Fitter and Hay 1991; Munns 1993). During this experiment, Panicum leaf senescence and death was observed to be initiated in the older (lower) leaves and progress toward the growing tip.

On the other hand, in the salt marsh dominant, Spartina alterniflora, despite many significant population differences in morphological characteristics prior to salinity stress, none of these characteristics provided a reliable means of identifying salt-tolerant or salt-sensitive genotypes. Spartina alterniflora possesses anatomical features, salt glands, that can excrete salt out of the leaf tissue and onto the leaf surface where it may be rinsed

from the leaf via rainfall or tidal flushing (Anderson 1974). Spartina alterniflora is also reported to be able to exclude salt (Smart and Barko 1980) or accumulate it in the leaf (Nestler 1977). Physiological responses to salt stress, such as the synthesis of compatible solutes, have also been reported for this species (Cavalieri and Huang 1979; 1981). Therefore, future research may show that physiological/biochemical differences among Spartina alterniflora populations are more important in explaining differences in salt tolerance than are population differences in plant morphology.

Both species showed decreased leaf expansion rates as salinities increased to stressful levels (Figure 3.3). Both species also displayed a similar range of leaf rolling response (24% to 26% reduction in projected leaf width) at their respective sublethal salinity levels (Table 3.1). However, leaf rolling in Spartina alterniflora at 35‰ was highly correlated with salinity tolerance and was able to explain 38% of the variation in lethal salinity level among populations, whereas leaf rolling in Panicum hemitomon at 4‰ was not correlated with salinity tolerance. The importance of leaf rolling as a mechanism of reducing water loss has been observed to vary across species. For example, in Spartina pectinata, leaf rolling has been shown to be of secondary importance to stomatal closure as a means of decreasing water loss (Heckathorn and DeLucia 1991). Furthermore, O'Toole and Moya (1978) reported that variation in the relationship of water potential and leaf rolling was observed to occur when screening widely divergent genotypes of rice. Our results indicate that leaf rolling in Spartina alterniflora does appear to be of some value in assessing salt tolerance. Populations that displayed less leaf rolling at 35‰ had higher lethal salinity levels than populations that were more tightly rolled.

In summary, this study has demonstrated that Gulf Coast populations of Panicum hemitomon and Spartina alterniflora display highly significant intraspecific variation in lethal salinity level and plant morphology. Morphological variables relevant to plant and leaf size may be useful for screening populations of Panicum hemitomon for superior salt

tolerance, whereas morphological differences among Spartina alterniflora populations appear to be of no value in assessing salt tolerance. It is suggested that in the salt marsh dominant, Spartina alterniflora, physiological or anatomical differences are likely to be more important in explaining differences in salt tolerance among populations than are variations in plant morphology. Principal components analysis was to shown to have potential value as an aid in identifying populations that display a suite of desirable traits, such as high salt tolerance in combination with high biomass production under salt stress, thereby facilitating selection of superior planting stocks that may be utilized in marsh creation and marsh restoration projects. Our future research will focus on investigating differences in physiological and biochemical responses in subsets of these populations, ranging from highly salt tolerant to poorly salt tolerant, when they are subjected to sublethal salinity levels.

Chapter 4

Investigations of Factors Associated with Intraspecific Variation in Salt Tolerance in Panicum hemitomon, Spartina patens and Spartina alterniflora. I. Plant CO_2 Assimilation, Water Use Efficiency, and Leaf Expansion

INTRODUCTION

Elevated salinity level is one of the potential causes of stress and ultimate death of wetland vegetation and therefore has been implicated as a factor affecting wetland loss (Boesch 1982; Mendelssohn et al. 1983; Salinas et al. 1986; Turner and Cahoon 1987; McKee and Mendelssohn 1989). Increases in salinity regime may be attributed to sea level rise, canalization, and storm surges associated with hurricanes and severe storm events (Turner et al. 1982; Salinas et al. 1986). Other changes in hydrology, such as the abandonment of a freshwater tributary, may lead to increased salinities in the surrounding marshes (Fisk 1955). It is well recognized that species differences in salinity tolerance (interspecific variation in salt tolerance) are largely responsible for the broad zonation of Louisiana coastal plant communities into what has been classified as fresh, intermediate, brackish and salt marsh plant communities located along the gradient from fresh to saline water (Chabreck 1972; 1981). In infrequently flooded coastal areas, such as high marsh areas and salt pans where salts accumulate to high levels at the soil surface as water evaporates from the soil, interspecific variation in salt tolerance can also be important in explaining small-scale vegetation patterns and dynamics associated with environmental heterogeneity in salinity (Bertness 1991; Shumway and Bertness 1994). However, the relative importance of intraspecific variation in salt tolerance in dominant coastal plant communities has not been intensively investigated (Silander 1979; Silander and Antonovics 1979; Pezeshki and DeLaune 1991; 1995) and may be important in understanding how these plant communities respond to changes in salinity regime.

Our previous research has shown that significant intraspecific variation in salt tolerance (lethal salinity level) exists in Gulf Coast populations of Panicum hemitomon, Spartina patens, and Spartina alterniflora, dominant emergent macrophytes of fresh, brackish, and salt marsh plant communities, respectively. The ability to identify plant populations that display varying degrees of salt tolerance within these species has important applied, ecological and theoretical significance. The selection of superior salt-tolerant planting stock for use in marsh creation/restoration projects may have applied value relevant to habitat and design considerations of a given project. The identification of genotypes of varying degree of salt tolerance within these coastal grasses also provides an excellent research opportunity in which to investigate mechanisms associated with differential salt tolerance. Through these investigations certain mechanisms or physiological/biochemical responses may be identified that contribute to our understanding of salt tolerance, and that furthermore may have potential value as markers for future screenings of plant populations for superior salt tolerance.

Panicum hemitomon Schult. is a fresh marsh grass that is distributed continuously along the coastal plain of the United States from New Jersey southward into Florida and westward along the Gulf coast into Texas. It is also found in some fresh marshes in Tennessee, as well as in South America (Godfrey and Wooten 1979). Throughout its range Panicum hemitomon is a common species in the fresh marsh plant community and in southeastern Louisiana it is the dominant emergent macrophyte of the coastal fresh marshes (Chabreck 1972).

Spartina patens (Ait.) Muhl. is a very important coastal grass species that is distributed continuously along the Atlantic and Gulf coasts of North America from Maine to south Texas. It is also occurs in the Yucatan peninsula, Cuba, the West Indies, and along some sandy shores of the Great Lakes (Mobberly 1956). Spartina patens has a wide ecological amplitude, ranging from dunes and swales to coastal intermediate and brackish marshes, where it is usually dominant (Duncan and Duncan 1987). In

Louisiana, Spartina patens is the dominant emergent macrophyte in the State's expansive brackish marshes and is the most frequently encountered grass species throughout the coastal zone (Chabreck 1970; 1972).

Spartina alterniflora Loisel. has been referred to as the most important grass species in coastal salt marshes (Pomeroy and Wiegert 1981; Duncan and Duncan 1987; Eleuterius 1990) and is distributed continuously along the Atlantic coast from New Foundland southward to Florida and westward along the Gulf coast to Texas (Godfrey and Wooten 1979). Spartina alterniflora is generally the dominant coastal salt marsh grass throughout its range, and in Louisiana it dominates the vast southern expanses of the State's coastal salt marshes (Chabreck 1972).

The goal of this series of investigations was to achieve a greater understanding of 1) plant photosynthetic and growth responses, 2) biomass partitioning, and 3) plant water relations and osmotic adjustment associated with intraspecific variation in salt tolerance within each of these three coastal marsh dominants. This manuscript is the first of a three-part series and reports the results of plant CO_2 assimilation, water use efficiency and leaf expansion when populations of varying degrees of salt tolerance within each of these species were subjected to a sublethal salinity excursion.

MATERIALS AND METHODS

Plant material

Population selection was based on our previous salinity screening research in which we subjected 19 to 25 populations of each of the three species to weekly stepwise salinity increases of 2‰, 5‰, and 10‰ for Panicum hemitomon, Spartina patens, and Spartina alterniflora, respectively (Chapters 2 and 3). All three species displayed highly significant intraspecific variation in lethal salinity level, which was defined as that salinity which resulted in 50% death of aboveground tissue. The range of lethal salinity levels for each species was as follows: Panicum hemitomon 7.6‰ - 12.0‰, Spartina patens 63‰ - 93‰, and Spartina alterniflora 83‰ - 115‰. Based on these results, two of the

most salt-tolerant genotypes, two genotypes of intermediate salt tolerance, and two of the least salt-tolerant genotypes were selected within each species. In this series the two highly salt-tolerant populations within each species are referred to as populations 1 and 2, the two populations of intermediate salinity tolerance as populations 3 and 4, and the two poorly salt-tolerant populations as populations 5 and 6. The corresponding alphabetic population codes for these selected populations, as used in the previous chapters on screening for intraspecific variation in salt tolerance, and their respective lethal salinity levels are as follows: Panicum hemitomon populations I (12‰), H (11.2‰), K (10‰), Q (9.6‰), S (7.6‰), B (7.6‰); Spartina patens populations O (93‰), E (89‰), P (83‰), I (81‰), K (66‰), B (63‰); Spartina alterniflora populations N (115‰), E (115‰), G (107‰), I (101‰), T (93‰), W (93‰).

Plant material was obtained by vegetatively propagating the selected genotypes from the stock populations, which had been maintained under non-saline conditions in a temperature controlled glasshouse for six to eight vegetative generations prior to the beginning of this experiment. Plants were propagated in a commercial potting mix (Jiffy Mix®; Chicago Illinois) and all pots were given equal amounts of half-strength (50%) Hoagland's nutrient solution (Hoagland and Arnon 1950) as needed to produce vigorous growth.

Experimental design

The experimental design for each species was a factorial randomized block design of six replicates, or blocks, that blocked on position within the growth chamber. The three species experiments were run sequentially, not simultaneously. For each species the following 6 x 2 x 2 factorial arrangement of main effects was tested: population effect (six populations ranging from high to low salinity tolerance), treatment effect (sublethal salinity level versus control at non-saline conditions), harvest effect (early harvest one week after exposure to sublethal salinity level and late harvest five weeks

after exposure to sublethal salinity level). This experimental design yielded a total of 144 experimental units for each species experiment.

All experiments were conducted in a temperature-controlled EGC walk-in growth chamber set to 16 hr daylength at 30 C and an 8 hr dark period at 24 C. Illumination (quantum flux density) inside the growth chamber was approximately 1200 - 1300 μmol m^{-2} s^{-1} at canopy height. Depending on the size stature of the plants, two to four young stems were planted per pot in Jiffy Mix. Six replicates of each of the treatment combinations were potted in 0.7 liter plastic pots equipped with bottom and side drainage holes. These pots were placed inside larger reservoir pots that contained the treatment bathing solution at the desired salinity. Treatment bathing solutions were maintained at a level of 4 to 5 cm below the soil surface. This allowed exchange of soil interstitial water with the treatment bathing solution without flooding the soil surface.

Each experiment consisted of two harvests: an early harvest one week after exposure to the designated sublethal salinity level and a late harvest after four additional weeks (five weeks total exposure to the sublethal salinity level). The following variables were measured during both harvests: leaf CO_2 assimilation and water use efficiency, leaf expansion rate, aboveground biomass (partitioned into live and dead components), belowground biomass (partitioned into root and rhizome components), leaf xylem pressure, leaf cation concentrations, and leaf proline and glycinebetaine concentrations. Additionally, during the early harvest determinations of leaf sugar concentrations (fructose, glucose, sucrose, and maltose) were also conducted. During the late harvest an index of leaf chlorophyll concentration was also determined. This manuscript is the first part of a three part series and reports the results of leaf CO_2 assimilation, chlorophyll index, water use efficiency and leaf expansion rate.

Salinity regime

The sublethal salinity level varied depending on the species as follows: Panicum hemitomon, 4‰; Spartina patens, 20‰; and Spartina alterniflora, 30‰. All

experimental units were initially maintained in a treatment bathing solution of half-strength Hoagland's nutrient solution at 0‰ salinity for a two week period following transplanting. Salinity was then increased stepwise over a relatively short time interval in half-strength Hoagland's until the targeted sublethal salinity level was reached using a commercial sea salts mix (Instant Ocean®; Aquarium Systems, Mentor, Ohio) with major ionic composition expressed as percentage of dry weight as follows: 46.9% Cl, 26.0% Na, 6.4% SO_4, 3.2% Mg, 1.0% Ca, and 0.9% K (Bidwell and Spotte 1985). Panicum hemitomon was subjected to a salinity increase of 2‰ for five days and then brought up to the sublethal salinity level of 4‰. Spartina patens was subjected to a salinity of 5‰ for two days, 10‰ for four days, and then brought up to the sublethal salinity level of 20‰. Spartina alterniflora was subjected to a salinity of 6‰ for three days, 15‰ for 5 days, and then brought up to the sublethal salinity level of 30‰.

Salinity increases for all species were accomplished by removing the pots from their reservoir containers and allowing them to drain for one hour. Pots were then flushed twice with two 350 ml aliquots of Instant Ocean solution at a salinity that was slightly higher than the desired step increase by slowly adding the solution to the soil surface and allowing it to drain through the soil and out the drainage holes over a period of two hours. Pots were placed back in the reservoir containers and a solution of half-strength Hoagland's with Instant Ocean at the targeted salinity level was slowly added to the soil surface in two 350 ml aliquots, which drained through the soil and collected in the reservoir containers. Salinity was rechecked for accuracy. Solution levels were checked daily. Controls were kept at 0‰ salinity in half-strength Hoagland's except for the Spartina alterniflora controls, which were provided with 1‰ sea salt in half-strength Hoagland's. All bathing solutions were drained and replaced at weekly intervals and salinities were checked twice weekly throughout each experiment.

Analytical techniques

Plant leaf photosynthesis (net CO_2 exchange rate) was measured on young, but fully expanded leaves (generally the second expanded leaf from the growing tip) during midday conditions (six to nine hours into the 16 hour photoperiod) on a randomly selected mature stem. Measurements were conducted with a portable infrared gas analyzer (Analytical Development Company, Herts, England; model LCA-2). The selected leaf was clamped into an ADC Parkinson leaf chamber and the difference in CO_2 concentration between inlet and outlet air was measured. Sampling air was taken outdoors at 5 m above the ground surface in order to obtain a relatively stable CO_2 concentration, which was led through an ADC air supply unit with silica columns to obtain a dry inlet airstream. The flow rate was held constant at 6.25 ml s^{-1} and measurements were conducted under light saturated photosynthetic conditions provided by a Kodak projector lamp at a quantum flux density of 2000 µmol m^{-2} s^{-1}. Gas exchange was determined on a per unit leaf area, and molar air flow, transpiration rate and CO_2 uptake were calculated according to von Caemmerer and Farquhar (1981). Water use efficiency was calculated as the ratio of µmol CO_2 fixed divided by mmol H_2O transpired (Griffiths 1993).

Leaf chlorophyll index was measured with a Minolta chlorophyll meter (model SPAD-502) on the second fully expanded leaf on five randomly selected stems per pot. Chlorophyll index is based on the inverse of the transmission of light at 650 nm and 940 nm through a leaf clamped in the measuring chamber, so that chlorophyll indices are linearly related with chlorophyll concentrations.

Leaf expansion was measured on terminal leaves that were neither newly emergent nor fully expanded, but in the range of one third to two thirds expanded. For Spartina patens and Spartina alterniflora, leaf length was measured from the tip of the terminal leaf to the ligule of the youngest expanded leaf over a time interval of three days. For Panicum hemitomon measurements of leaf expansion were taken from the tip of the

terminal leaf to a fixed point (the rim of the pot) over a time interval of three days, and thus represent a composite of expansion of the terminal leaf in addition to any stem elongation below the upper ligule that occurred during that time interval. These methods have been used successfully with other grasses and produced results which parallel changes in aboveground biomass and photosynthesis (Hester and Mendelssohn 1990; Koch et al. 1990).

Data analysis

Data was analyzed as a factorial randomized block design. Analysis of variance (ANOVA) was used to test for significant main effects and interactions using SAS (SAS 1989; Steel and Torrie 1980). A significance (alpha) level of 0.05 was used for all analyses unless otherwise stated. Single degree of freedom contrasts were used to make a priori comparisons between populations of different degrees of salt tolerance. All data were tested for meeting the assumptions of normality and homogeneity of variance by using a combination of the Shapiro-Wilk test statistic for tests of normality and the Bartlett test for tests of homogeneity of variance (SAS 1989). Data that did not meet these assumptions were transformed until assumptions were met.

RESULTS

Panicum hemitomon

Prolonged exposure to 4‰ salinity had a cumulative adverse effect on several of the treatment experimental units by the late harvest and resulted in insufficient green leaf tissue for measurements of leaf CO_2 assimilation, water use efficiency, and leaf xylem pressure. Population 5 (poorly salt tolerant) displayed severe browning on four of its six treatment experimental units. One experimental unit of population 6 (poorly salt-tolerant), and one experimental unit of population 2 (highly salt tolerant) also lacked sufficient green leaf tissue for measurements of these variables. Quantification of live and dead aboveground biomass is provided in the next chapter (Chapter 5, Part II).

Net CO_2 assimilation showed significant harvest, treatment and population main effects and significant harvest x treatment and treatment x population interactions. Inspection of Figure 4.1 shows that controls consistently maintained greater CO_2 assimilation rates than treatments, and that both controls and treatments showed a decrease in CO_2 assimilation rates in the late harvest (possibly due to crowding), but that the salinity treatments decreased more, leading to the significant harvest x treatment interaction. Contrasts within treatments showed that the highly salt-tolerant populations (populations 1 and 2) had significantly greater rates of CO_2 assimilation (23.2 and 22.4 $\mu mol\ m^{-2}\ s^{-1}$, respectively) than the poorly salt-tolerant populations (14.5 and 21.7 $\mu mol\ m^{-2}\ s^{-1}$ for populations 5 and 6, respectively) in the early harvest, but in the late harvest this difference was significant only at $P \leq 0.10$ (Figure 4.1). Conversely, contrasts within controls showed no significant differences between highly and poorly salt-tolerant populations in the early harvest, but significantly greater CO_2 assimilation rates in the late harvest poorly salt-tolerant controls (Figure 4.1). The significant population x treatment interaction may be seen as a greater decrease in CO_2 assimilation rate between salinity treatments and their corresponding controls in the poorly salt-tolerant populations compared to the highly salt-tolerant populations (Figure 4.1). Highly salt-tolerant populations in the early and late harvests had treatment photosynthetic rates that were 85% and 67% of controls, respectively, compared to 63% and 30% for the poorly salt-tolerant populations.

Measurements of late harvest leaf chlorophyll index revealed significant treatment and population main effects and a significant treatment x population interaction. Contrasts within treatments showed that the highly salt-tolerant populations had relatively greater chlorophyll indices (37 and 30 for populations 1 and 2, respectively) than the poorly salt-tolerant populations (13 and 28 for populations 5 and 6, respectively), whereas contrasts within controls were not significant (Figure 4.2). Also, differences between treatments and controls within populations were significant only for the two

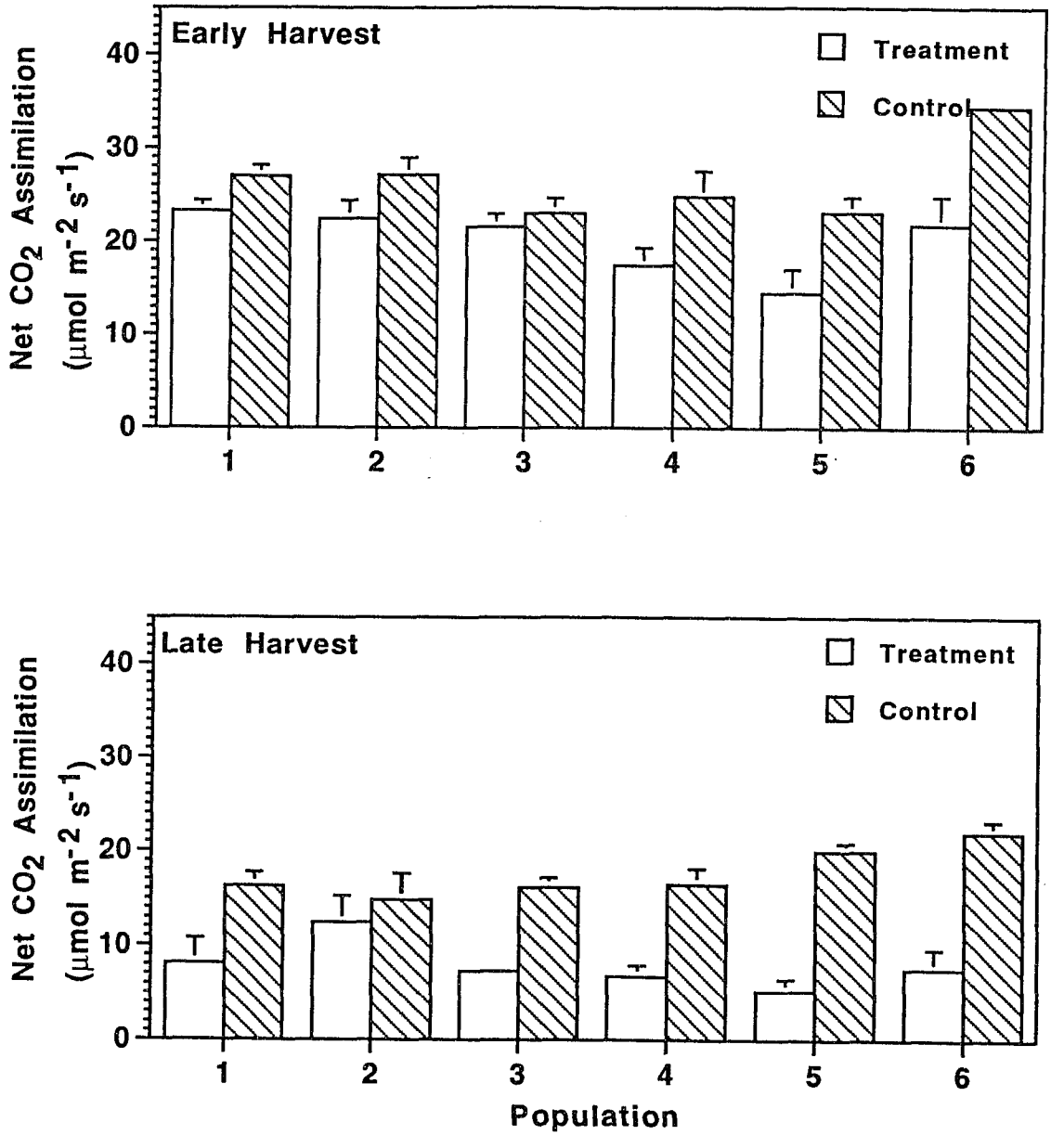

Figure 4.1. Mean (± std err) leaf net CO_2 assimilation rate (μmol m^{-2} s^{-1}) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Panicum hemitomon when subjected to a sublethal salinity excursion of 4‰ for one week (early harvest) and five weeks (late harvest); n=6 except for the following late harvest salinity treatments: n=5 for population 2, n=2 for population 5 and n=5 for population 6; $LSD_{0.05}$=5.48; MSE=20.46.

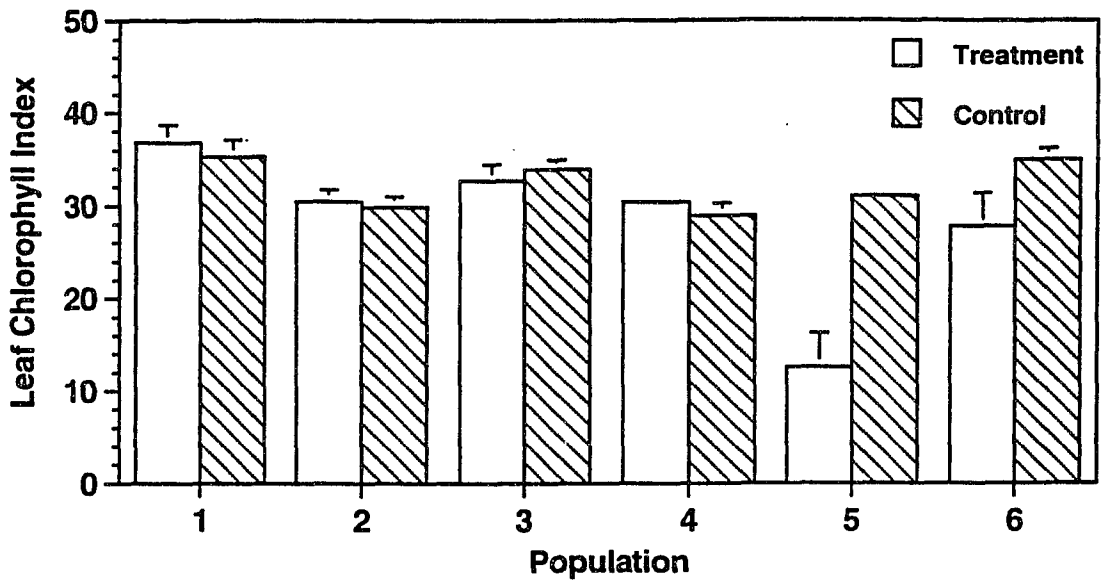

Figure 4.2. Mean (± std err) leaf chlorophyll index in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Panicum hemitomon when subjected to a sublethal salinity excursion of 4‰ for five weeks (late harvest); n=6, $LSD_{0.05}$=5.02, MSE=18.83.

poorly salt-tolerant populations (populations 5 and 6), which showed significantly lower leaf chlorophyll indices in the treatments relative to the controls (Figure 4.2).

Water use efficiency (WUE, expressed as μmol CO_2 fixed per mmol H_2O transpired) displayed significant harvest, treatment, and population main effects and significant harvest x treatment and treatment x population interactions (Figure 4.3). Contrasts between highly salt-tolerant and poorly salt-tolerant populations showed no significant differences within controls for either harvest, nor within treatments in the early harvest. However, contrasts within treatments in the late harvest did show significantly greater water use efficiencies in the highly salt-tolerant populations (2.2 and 2.8 μmol CO_2 $mmol^{-1}$ H_2O for populations 1 and 2, respectively), indicating that they were fixing proportionately more CO_2 per H_2O transpired than the poorly salt-tolerant populations (1.2 and 1.8 μmol CO_2 $mmol^{-1}$ H_2O for populations 5 and 6, respectively; Figure 4.3).

Leaf expansion rate showed significant harvest, treatment and population main effects, in addition to significant harvest x treatment and harvest x population interactions (Figure 4.4). Leaf expansion rates were generally less in the late harvest than in the early harvest and differences between treatments and their respective controls (within populations) tended to not be significant for the late harvest, whereas in the early harvest, controls had consistently greater rates of leaf expansion than the treatments (Figure 4.4). Contrasts failed to detect any significant differences between highly salt-tolerant populations and poorly salt-tolerant populations within treatments or controls of the early harvest. In the late harvest, the poorly salt-tolerant controls actually had significantly greater leaf expansion rates than the highly salt-tolerant controls. Although late harvest contrasts within treatments detected no significant differences between highly salt-tolerant (populations 1 and 2) and poorly salt-tolerant populations (populations 5 and 6), population 5 alone had a very reduced leaf expansion rate that was significantly lower than all other late harvest populations (Figure 4.4).

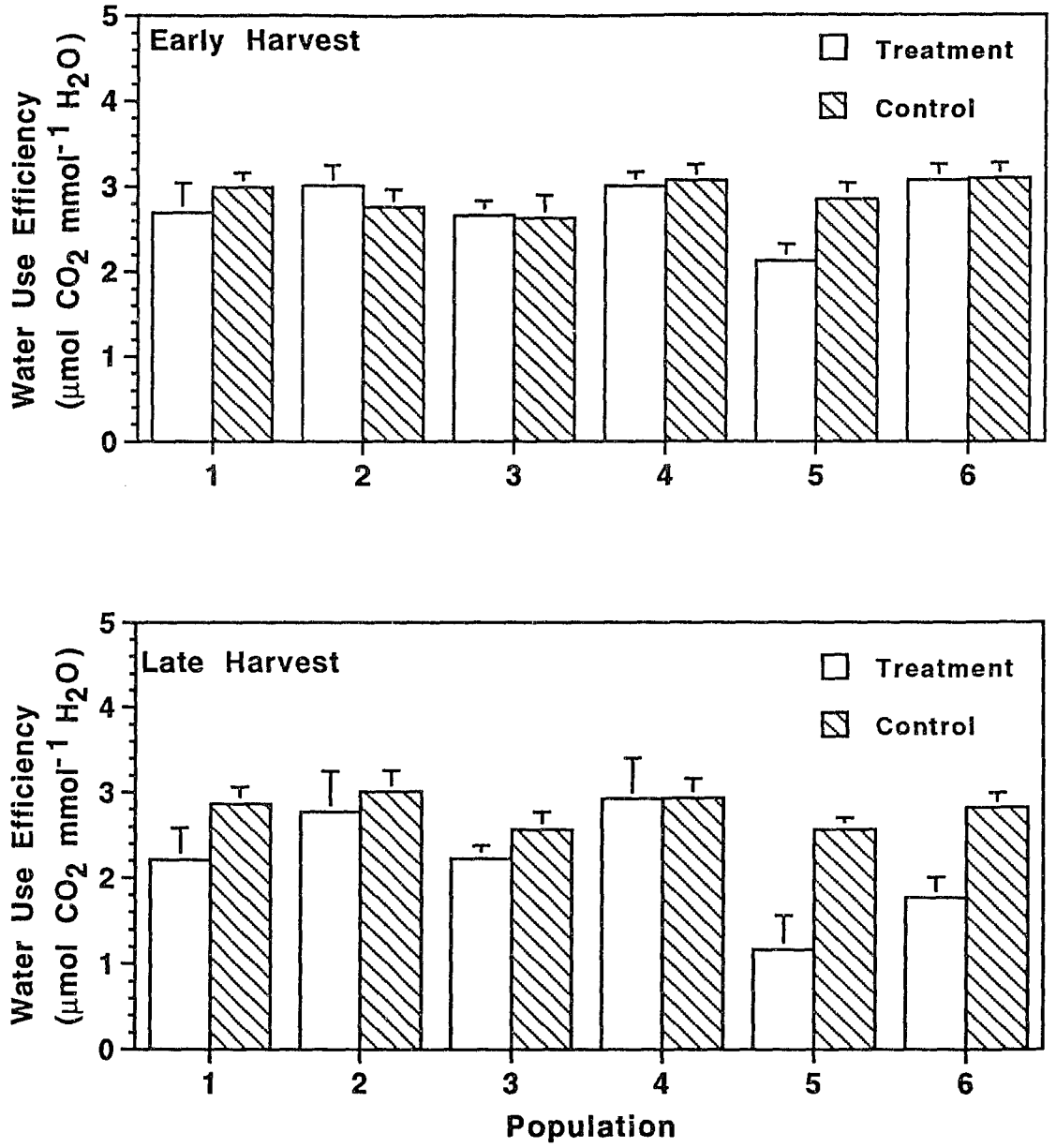

Figure 4.3. Mean (± std err) leaf water use efficiency (μmol CO_2 $mmol^{-1}$ H_2O) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Panicum hemitomon when subjected to a sublethal salinity excursion of 4‰ for one week (early harvest) and five weeks (late harvest); n=6 except for the following late harvest salinity treatments: n=5 for population 2, n=2 for population 5 and n=5 for population 6; $LSD_{0.05}$=0.671; MSE=0.296.

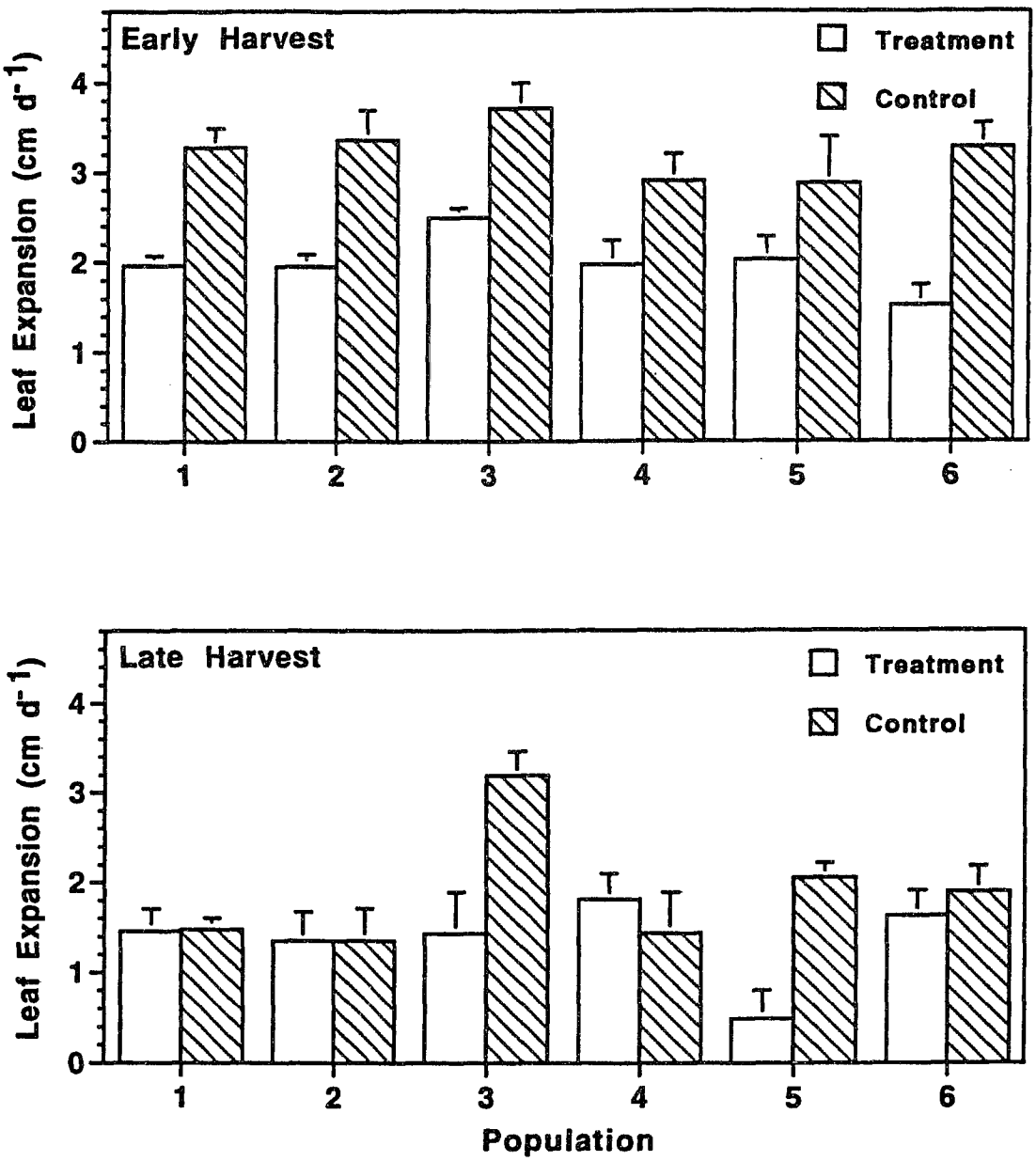

Figure 4.4. Mean (± std err) leaf expansion rate (cm day^{-1}) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Panicum hemitomon when subjected to a sublethal salinity excursion of 4‰ for one week (early harvest) and five weeks (late harvest); n=6, $LSD_{0.05}$=0.829, MSE=0.513).

Spartina patens

Net CO_2 assimilation rate showed no significant main effects or interactions for Spartina patens (Figure 4.5). Contrasts within controls showed no significant differences between highly salt-tolerant and poorly salt-tolerant populations. Within treatments, there was a tendency for the early harvest salt-tolerant populations to have greater CO_2 assimilation rates than the poorly salt-tolerant populations ($P \leq 0.07$), and a contrast between population 1 versus population 6 did show population 1 to have a significantly greater net CO_2 assimilation rate (42.0 μmol m^{-2} s^{-1}) than population 6 (29.5 μmol m^{-2} s^{-1} ; Figure 4.5).

Late harvest leaf chlorophyll index showed significant treatment and population effects with the treatments averaging greater chlorophyll indices (36.7) than the controls (28.2; Figure 4.6). Contrasts showed that highly salt-tolerant populations had significantly greater chlorophyll indices than poorly salt-tolerant populations in both treatments and controls (Figure 4.6). Treatment chlorophyll indices for populations 1 and 2 were 41 and 37, respectively, compared to 34 and 36, respectively for populations 5 and 6 (Figure 4.6).

Water use efficiency displayed a significant harvest main effect and a significant harvest x population interaction (Figure 4.7). Water use efficiencies for the highly salt-tolerant populations remained very similar between harvests, whereas other populations, particularly the poorly salt-tolerant populations, displayed increased water use efficiencies in the late harvest. Contrasts failed to show any significant differences in either harvest between highly salt-tolerant populations and poorly salt-tolerant populations within treatments or controls (Figure 4.7).

Rates of leaf expansion showed significant harvest and treatment main effects and a significant harvest x treatment interaction (Figure 4.8). Leaf expansion rates in the early harvest were consistently greater in controls than in treatments, whereas in the late harvest control rates of leaf expansion decreased significantly (possibly due to self

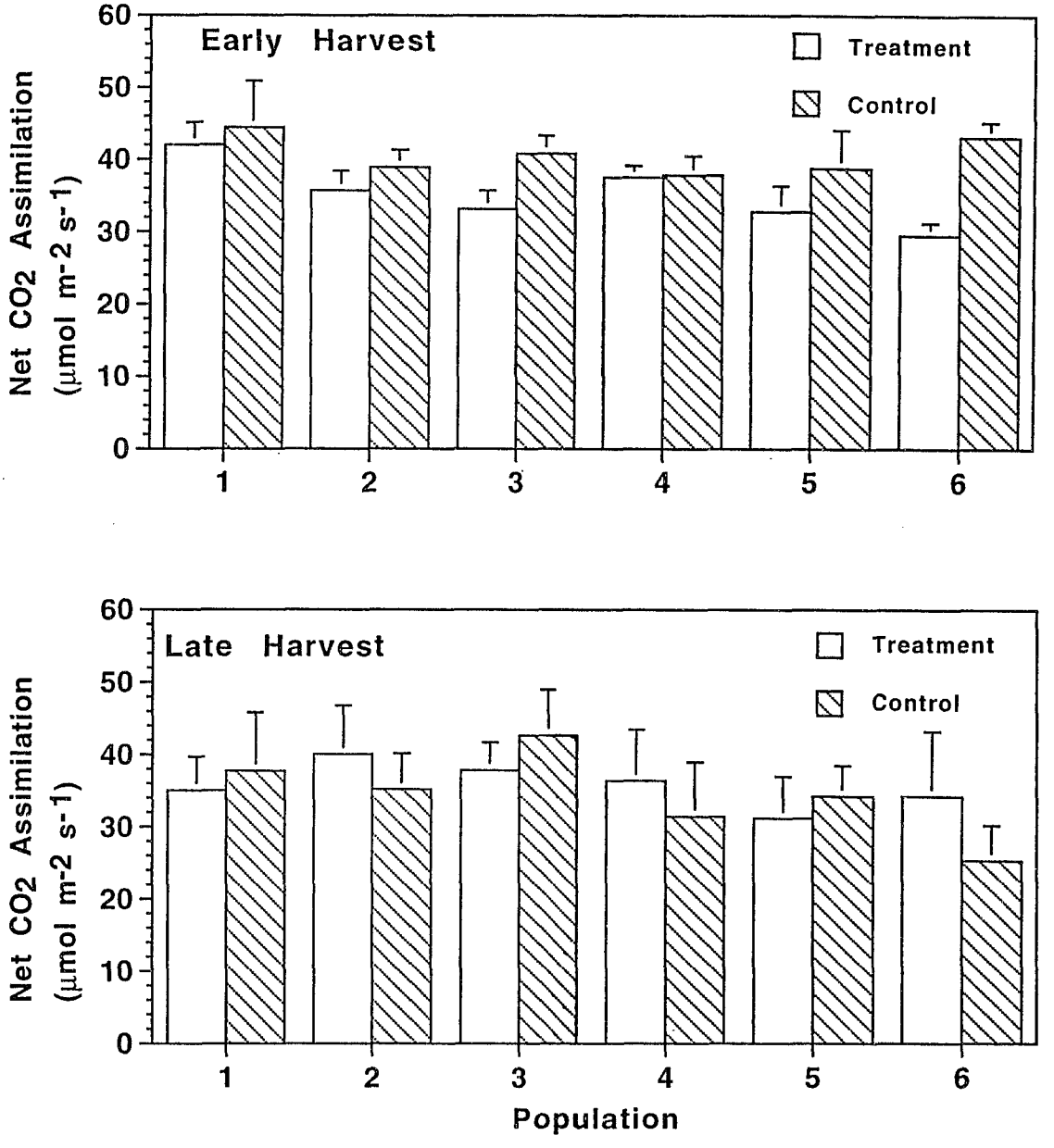

Figure 4.5. Mean (± std err) leaf net CO_2 assimilation rate ($\mu mol\ m^{-2}\ s^{-1}$) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Spartina patens when subjected to a sublethal salinity excursion of 20‰ for one week (early harvest) and five weeks (late harvest); n=6, $LSD_{0.05}$=12.28, MSE=112.2.

Figure 4.6. Mean (± std err) leaf chlorophyll index in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Spartina patens when subjected to a sublethal salinity excursion of 20‰ for five weeks (late harvest); n=6, $LSD_{0.05}$=2.88, MSE=6.19.

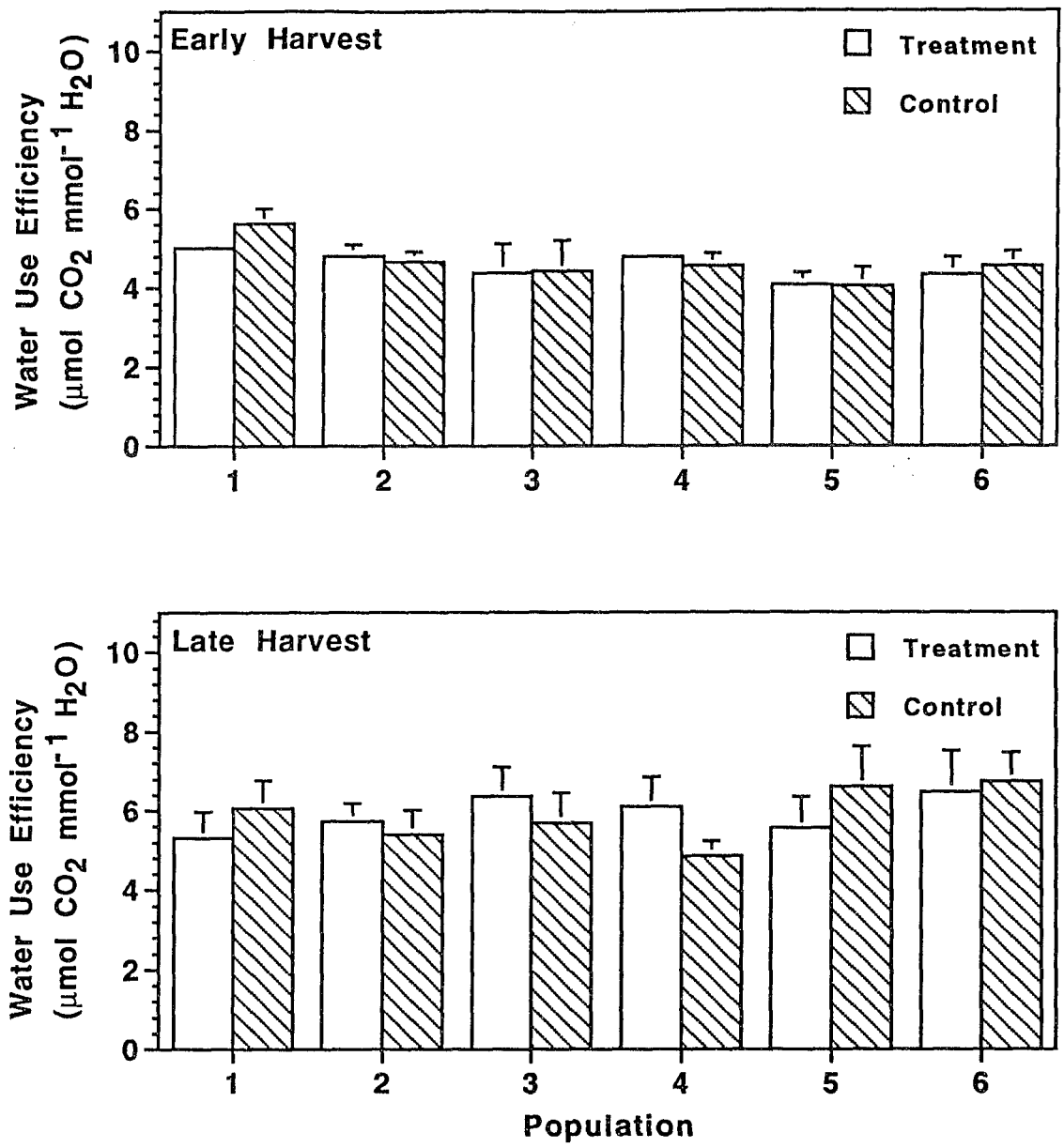

Figure 4.7. Mean (± std err) leaf water use efficiency (μmol CO_2 $mmol^{-1}$ H_2O) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Spartina patens when subjected to a sublethal salinity excursion of 20‰ for one week (early harvest) and five weeks (late harvest); n=6, $LSD_{0.05}$=1.33, MSE=1.32.

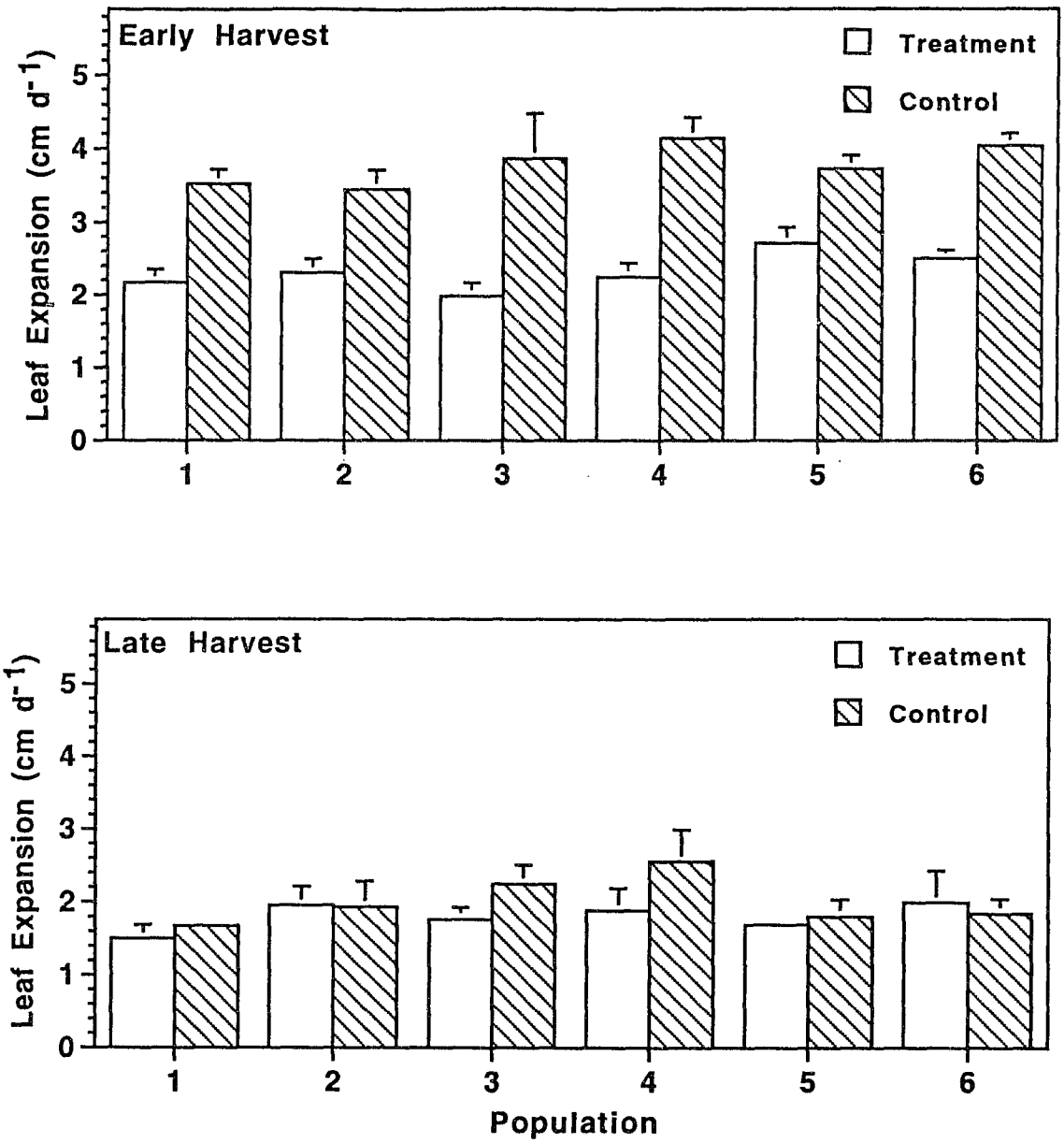

Figure 4.8. Mean (± std err) leaf expansion rate (cm day^{-1}) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Spartina patens when subjected to a sublethal salinity excursion of 20‰ for one week (early harvest) and five weeks (late harvest); n=6, $LSD_{0.05}$=0.754, MSE=0.434.

shading) while treatment rates decreased only slightly. Contrasts between highly salt-tolerant and poorly salt-tolerant populations failed to detect any significant differences within treatments or controls (Figure 4.8).

Spartina alterniflora

Net CO_2 assimilation rate showed significant treatment and population main effects in addition to significant harvest x treatment and treatment x population interactions (Figure 4.9). These interactions can be seen as a tendency for some controls to show decreased photosynthetic rates in the late harvest compared to the early harvest, whereas some treatments showed increased photosynthetic rates in the late harvest compared to the early harvest (Figure 4.9). Contrasts revealed that in the early harvest the highly salt-tolerant populations had significantly greater net CO_2 assimilation rates (average of 20 μmol m^{-2} s^{-1}) than poorly salt-tolerant populations (average of 8.6 μmol m^{-2} s^{-1}) within treatments, but not within controls. In the late harvest, neither treatments nor controls showed significant differences between highly and poorly salt-tolerant populations (Figure 4.9).

Leaf chlorophyll had significant treatment and population effects, as well as a significant treatment x population interaction (Figure 4.10). Contrasts within treatments showed that the highly salt-tolerant populations had significantly lower chlorophyll indices (43 and 41, respectively) than the poorly salt-tolerant populations (55 and 44, respectively), whereas within controls there were no significant differences. Inspection of Figure 4.10 shows that there was a tendency for the intermediate and poorly salt-tolerant populations to have treatment chlorophyll values exceed those of the controls; this difference was significant for populations 3, 4, and 6.

Water use efficiency displayed significant harvest and treatment main effects (Figure 4.11). Early harvest water use efficiencies were less than the late harvest, and controls had greater water use efficiencies than the salinity treatments. Early harvest water use efficiencies averaged 5.4 and 4.1 μmol CO_2 $mmol^{-1}$ H_2O for controls and

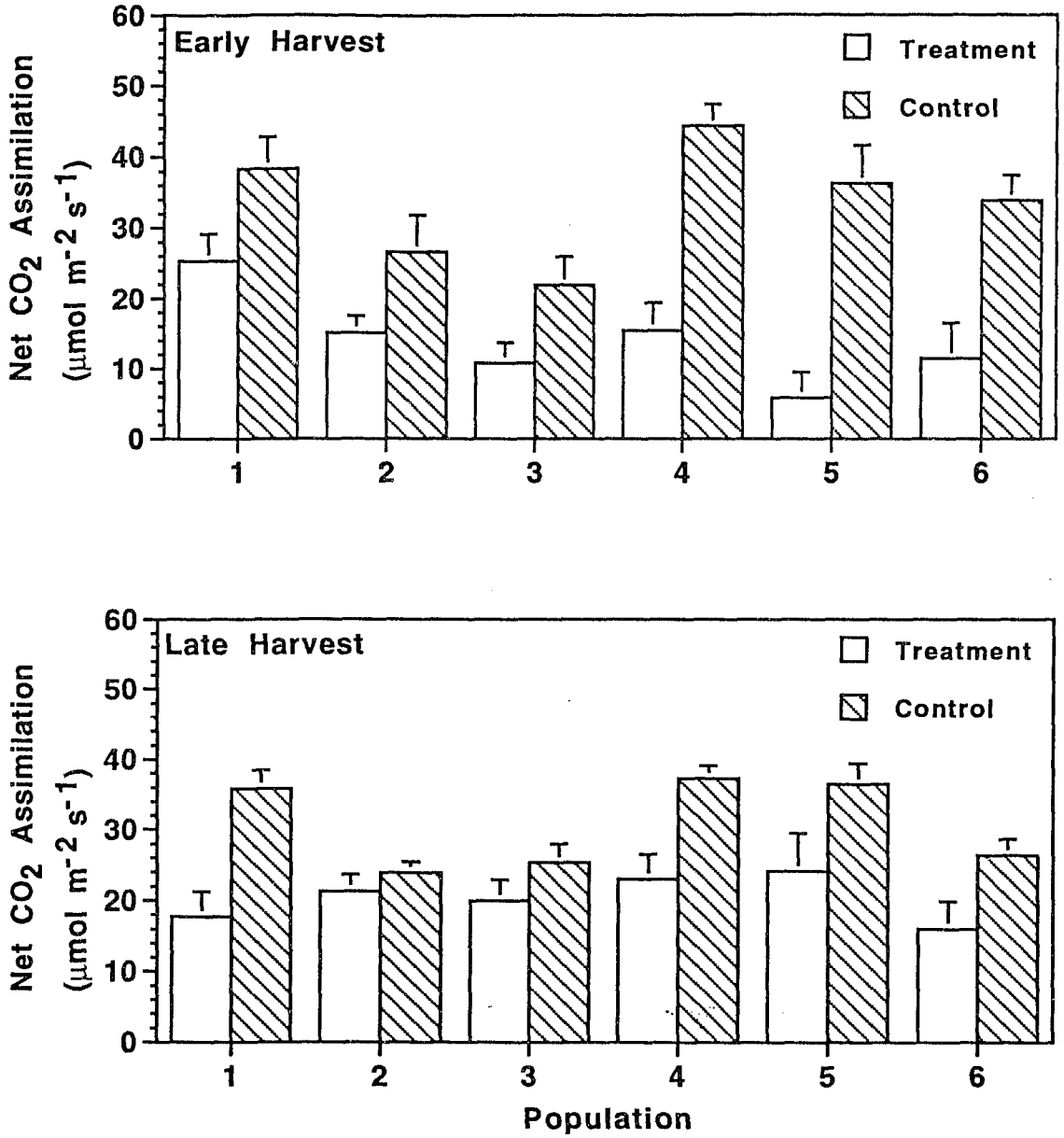

Figure 4.9. Mean (± std err) leaf net CO_2 assimilation rate (μmol m^{-2} s^{-1}) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Spartina alterniflora when subjected to a sublethal salinity excursion of 30‰ for one week (early harvest) and five weeks (late harvest); n=6, $LSD_{0.05}$=9.76, MSE=72.75.

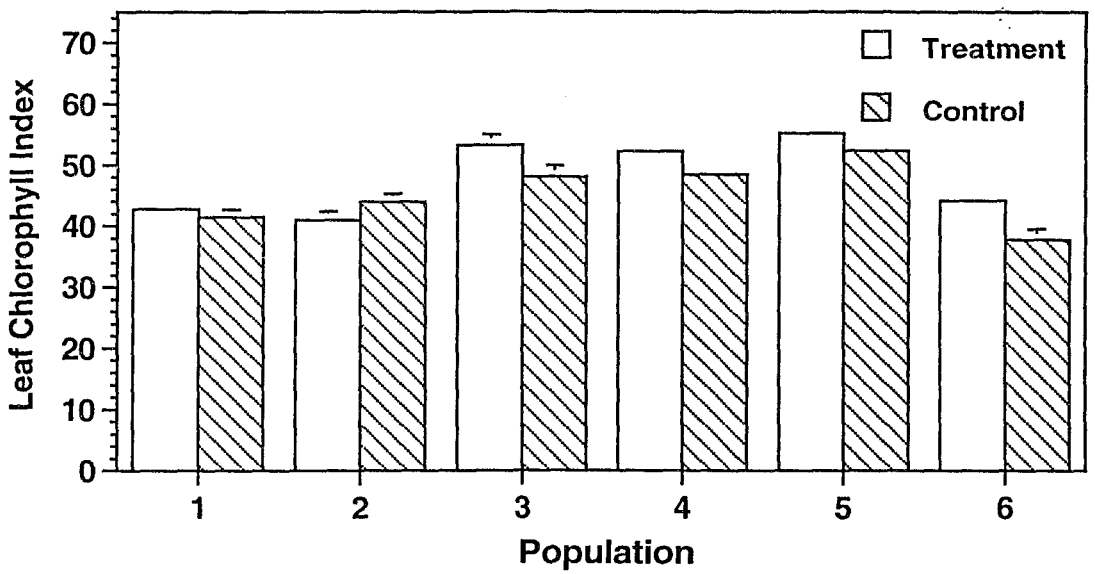

Figure 4.10. Mean (± std err) leaf chlorophyll index in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Spartina alterniflora when subjected to a sublethal salinity excursion of 30‰ for five weeks (late harvest); n=6, $LSD_{0.05}$=3.75, MSE=10.52.

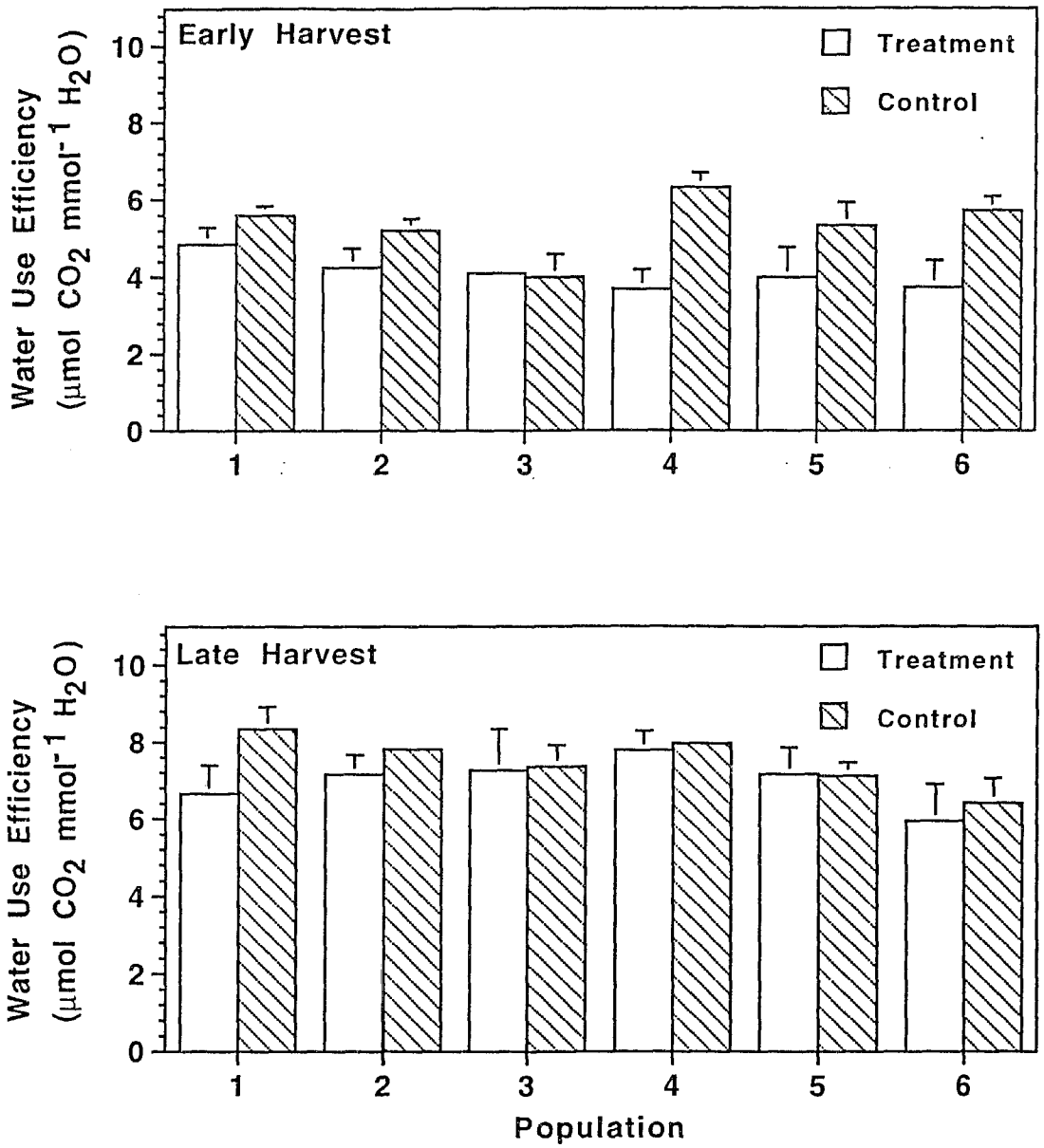

Figure 4.11. Mean (± std err) leaf water use efficiency (µmol CO_2 $mmol^{-1}$ H_2O) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Spartina alterniflora when subjected to a sublethal salinity excursion of 30‰ for one week (early harvest) and five weeks (late harvest); n=6, $LSD_{0.05}$=1.63, MSE=1.80.

treatments, respectively, compared to respective late harvest values of 7.5 and 7.0 μmol CO_2 $mmol^{-1}$ H_2O (Figure 4.11). Contrasts (within controls or treatments) failed to detect any significant differences between highly salt-tolerant and poorly salt-tolerant populations in either the early or late harvest despite the tendency for population 6 to have lower water use efficiency values (Figure 4.11).

Leaf expansion rate displayed significant treatment and population main effects and a significant treatment x population interaction (Figure 4.12). In both early and late harvests, controls had significantly greater rates of leaf expansion than treatments. Contrasts within treatments revealed that the highly salt-tolerant populations had significantly greater rates of leaf expansion than the poorly salt-tolerant populations during the early harvest, but not during the late harvest. Contrasts within controls showed no significant differences between highly salt-tolerant and poorly salt-tolerant populations during either the early or late harvests (Figure 4.12).

DISCUSSION

Net CO_2 assimilation data of all three species indicated that the more salt-tolerant populations were able to maintain higher rates of photosynthesis per unit leaf area than the poorly salt-tolerant populations one week after being subjected to a sublethal salinity excursion. However, with the exception of Panicum ($P \leq 0.10$), late harvest data showed that salt-tolerant populations did not necessarily maintain higher rates of net CO_2 assimilation than other populations when the salinity stress extended over a five-week period. Population differences in salt tolerance were not evidenced in controls maintained under non-stressful conditions with the exception of the late harvest controls of Panicum hemitomon, and in this case it was the poorly salt-tolerant controls that displayed the greater photosynthetic rates, not the highly salt tolerant controls (Figure 4.1).

In the late harvest, differences between treatments and controls in photosynthetic response were generally lessened in Spartina patens and Spartina alterniflora, whereas

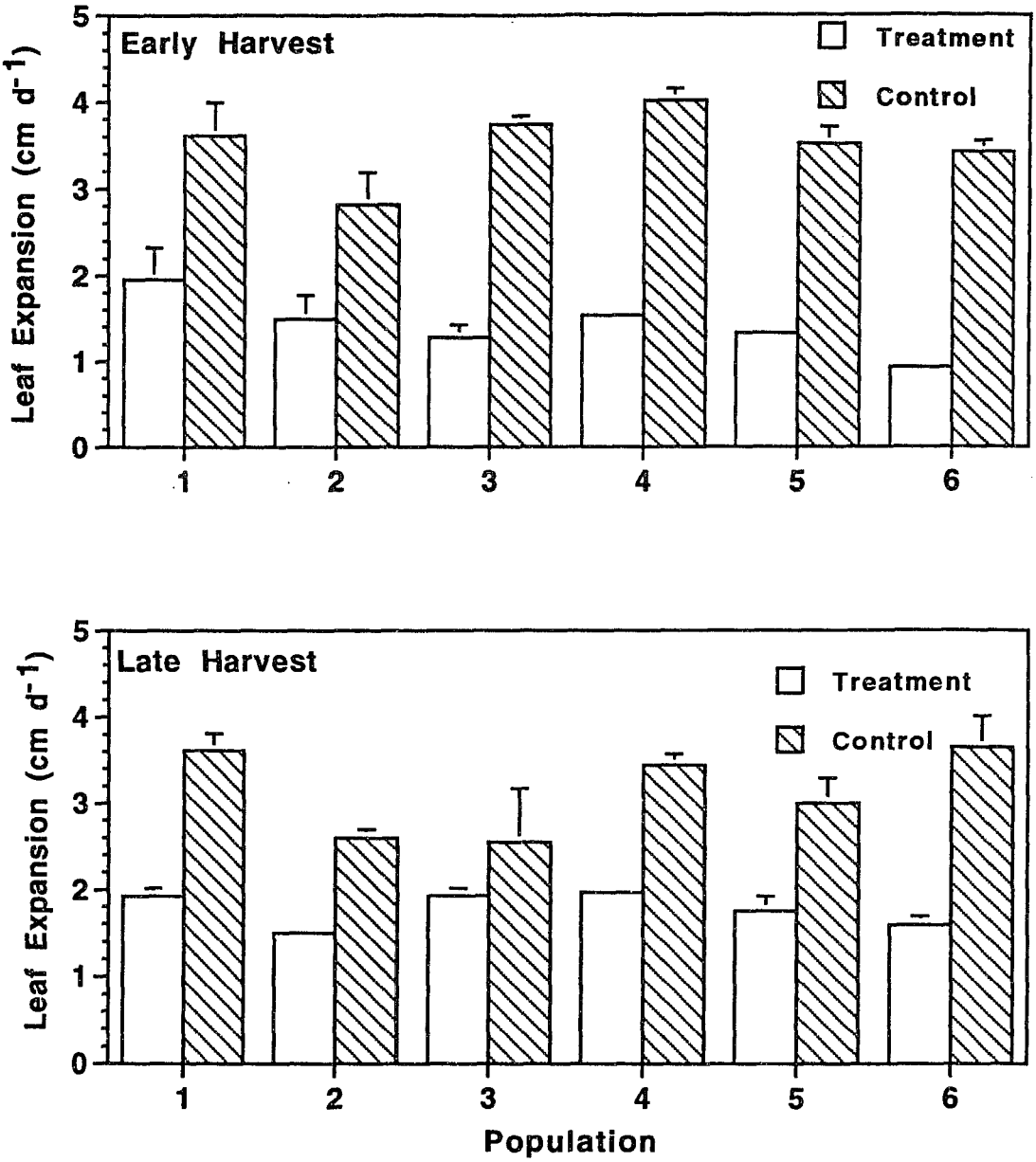

Figure 4.12. Mean (± std err) leaf expansion rate (cm day^{-1}) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Spartina alterniflora when subjected to a sublethal salinity excursion of 30‰ for one week (early harvest) and five weeks (late harvest); n=6, $LSD_{0.05}$=0.691, MSE=0.367.

treatment effects on Panicum hemitomon photosynthesis became more pronounced. This is likely a reflection of the inability of Panicum hemitomon to exclude salt, such that continued exposure to saline conditions had a cumulative negative effect, whereas in the two Spartinas, longer exposure to a sublethal salinity stress did not necessarily reduce photosynthetic response further than what occurred after one week, possibly because of their ability to exclude or secrete salt (Anderson 1974; Smart and Barko 1980; Bradley and Morris 1991). The lack of sufficient green leaf tissue to conduct late harvest measurements on several experimental units of Panicum illustrates this cumulative effect of salt stress on mature leaf tissue (Munns and Termaat 1986). Furthermore, decreases in photosynthetic rate with prolonged salt stress may be related to reduced sink activity due to tissue senescence and slowed new growth (Herold 1980).

Late harvest chlorophyll indices generally supported the early harvest photosynthetic response results in Panicum hemitomon and Spartina patens, with the more salt-tolerant populations having relatively more leaf chlorophyll than the poorly salt-tolerant populations when subjected to sublethal salinity stress. In Panicum it is possible that differences in leaf chlorophyll concentration were at least partly responsible for the observed continued differences in late harvest photosynthetic responses since rates of CO_2 assimilation can be limited by low chlorophyll concentration (Salisbury and Ross 1978). However, in Spartina alterniflora, it was the highly salt-tolerant populations that had significantly greater photosynthetic rates in the early harvest despite the fact that the poorly salt-tolerant populations had higher chlorophyll indices in the late harvest (when there were no longer significant population differences in photosynthetic rates). This indicates that in Spartina alterniflora, and possibly in the other species as well, the poorly salt-tolerant populations had some factor(s) other than leaf chlorophyll which limited photosynthesis under salt stress. Decreases in net CO_2 assimilation under salt stress may be attributed to stomatal control factors (Longstreth and Strain 1977; Percy and Ustin 1984) and non-stomatal, or metabolic factors (Longstreth and Nobel

1979; Walker et al 1982; Longstreth et al. 1984). Seemann and Critchley (1985) reported that reduced photosynthesis in salt-stressed bean (Phaseolus vulgaris) showed implications of stomatal limitations leading to decreased intercellular CO_2, as well as non-stomatal effects of the salt stress on photosynthesis, such as decreased quantum efficiency for CO_2 uptake, and an apparent effect on ribulose bisphosphate carboxylase activity.

Treatment chlorophyll indices in Spartina patens showed that the treatments frequently had higher chlorophyll concentrations than their respective controls, and may indicate that the sublethal salinity level of 20‰ was actually not that stressful to Spartina patens. It may further indicate that the controls were not growing under optimum conditions since no salt was added to the Spartina patens control bathing solutions as was done for Spartina alterniflora controls (1‰ sea salts were added). Pezeshki (1991) concluded that for two populations of Spartina patens identified as being of different salinity tolerances, gas exchange responses were quite similar at salinities less than 25‰, at which point the more salt-tolerant population displayed less of a decrease in carbon assimilation than the more poorly salt-tolerant population, although differences in water use efficiencies were not apparent.

In this study, water use efficiencies at sublethal salinity levels in Spartina patens and Spartina alterniflora did not detect known population differences in salt tolerance. However in the fresh marsh dominant, Panicum hemitomon, the poorly salt-tolerant populations did have significantly lower water use efficiencies than the other populations in the late harvest. Therefore, not only were the poorly salt-tolerant Panicum populations experiencing reduced photosynthetic rates, the photosynthesis that was occurring was expending relatively more water than the highly salt-tolerant populations.

Spartina alterniflora leaf expansion rates within early harvest treatments were significantly greater in the highly salt-tolerant populations than the poorly salt-tolerant populations. Otherwise, leaf expansion rates did not reflect population differences in salt

tolerance despite the fact that all three species showed a decrease in leaf expansion in the early harvest salinity treatments. Munns (1993; and reference therein) reported that upon the onset of salinity stress, leaf expansion is inhibited before a decrease in photosynthesis is evident, which is contrary to a common dogma in salinity research which states that photosynthesis is first decreased, which then limits growth (Munns 1993). However, it needs to be stated that time scales cited in this argument of leaf expansion being inhibited first may be as short as minutes (Yeo et al. 1991). Since both growth (leaf expansion) and photosynthesis in this study were already reduced in the early harvest (one week), there is no way of knowing which process was reduced first. Nonetheless, in this study, it appears that leaf photosynthetic response was more sensitive than leaf expansion rates in detecting known population differences in salt tolerance after one week of salinity stress. Similarly, Rawson et al. (1988) found that in wheat and barley genotypes of known salinity tolerance, leaf expansion rates of single leaves 10 days after salt application were poor indicators of known salt tolerance; only whole-plant leaf area increases were correlated with salt tolerance.

In summary, previous differentiation of Panicum hemitomon, Spartina patens and Spartina alterniflora populations as highly salt-tolerant to poorly salt-tolerant based on lethal salinity level was most clearly associated with sublethal salinity level plant photosynthetic response in Panicum hemitomon. It may be that for Spartina alterniflora and particularly Spartina patens, the sublethal salinity level was not high enough to induce good population separation in terms of photosynthesis and growth responses. Also, the Spartinas showed some recovery in the late harvest, whereas Panicum generally did not. These differences in severity of response between the Spartinas and Panicum may be due in part to the ability of both Spartina patens and Spartina alterniflora to secrete and exclude salt. For Panicum hemitomon, a salinity of 4‰ for five weeks had a cumulative effect that became near lethal for several of the poorly salt-tolerant experimental units. Species displayed differences concerning which response were most

useful to differentiate populations of known salt tolerance. Water use efficiency could differentiate populations of known salt tolerance only in Panicum hemitomon after five weeks of salinity stress. Leaf expansion rates were reduced below controls, but generally not in manner consistent with salt tolerance, with the exception of Spartina alterniflora one week after salinity stress. Leaf net CO_2 assimilation rate appeared to be the most sensitive to detect known population differences in salt tolerance one week after exposure to sublethal salinity. In Spartina patens the strength of population differentiation with various responses was generally less than observed in the other two species.

Chapter 5

Investigations of Factors Associated with Intraspecific Variation in Salt Tolerance in Panicum hemitomon, Spartina patens and Spartina alterniflora. II. Biomass Partitioning

INTRODUCTION

Our previous research has shown that significant intraspecific variation in salt tolerance (lethal salinity level) exists in Gulf Coast populations of Panicum hemitomon, Spartina patens, and Spartina alterniflora, dominant emergent macrophytes of fresh, brackish, and salt marsh plant communities, respectively. The identification of populations of varying degree of salt tolerance within each of these coastal grasses has provided an excellent research opportunity in which to investigate mechanisms and plant responses associated with differential salt tolerance in these important coastal grass species. This series of investigations was conducted at a sublethal salinity level selected for each species on populations previously classified as highly salt tolerant to poorly salt tolerant based on incremental salinity increases until 50% death of aboveground tissue was observed.

In the first part of this series results of plant photosynthesis and growth responses were presented. In all three species, plant photosynthetic rates in populations previously classified as highly salt-tolerant were greater than those of populations classified as poorly salt tolerant one week after exposure to sublethal salinity levels. Panicum hemitomon continued this trend of the highly salt-tolerant populations having higher photosynthetic rates into the late harvest (five weeks), whereas Spartina patens and Spartina alterniflora did not. Water use efficiencies in late harvest Panicum populations were also greater in the more salt-tolerant populations compared to the two Spartina species, which did not show significant population differences. Leaf expansion rates generally followed similar patterns to those of photosynthesis, but significant

differences between highly salt-tolerant and poorly salt-tolerant populations were not detected in any of the three species. Spartina patens in general showed the least amount of detectable differentiation between populations of known varying lethal salinity levels when subjected to a sublethal salinity excursion.

This manuscript is the second of this three part series and reports results of plant biomass partitioning in Panicum hemitomon, Spartina patens and Spartina alterniflora when populations of varying degrees of salt tolerance were subjected to a sublethal salinity level selected for each species. Biomass responses are useful in screening genotypes for salinity tolerance since biomass integrates other plant growth responses, such as photosynthesis and leaf expansion, into a cumulative growth response of the individual under salinity stress.

MATERIALS AND METHODS

Plant material

Population selection was based on our previous salinity screening research in which we identified intraspecific variation in lethal salinity level in each of these three species as described in Part I (Chapter 4) of this series. Based on these results, we selected two of the most salt-tolerant genotypes, two genotypes of intermediate salt tolerance, and two of the least salt-tolerant genotypes within each species. In this series we refer to the highly salt-tolerant populations within each species as populations 1 and 2, the two populations of intermediate salinity tolerance as populations 3 and 4, and the two poorly salt-tolerant populations as populations 5 and 6. The corresponding alphabetic population code for these selected populations, as used in the previous research on screening for intraspecific variation in salt tolerance is described in Part I. The respective lethal salinity levels for populations 1 through 6 of each species are as follows: Panicum hemitomon populations 1 (12‰), 2 (11.2‰), 3 (10‰), 4 (9.6‰), 5 (7.6‰), 6 (7.6‰); Spartina patens populations 1 (93‰), 2 (89‰), 3 (83‰), 4 (81‰),

5 (66‰), 6 (63‰); Spartina alterniflora populations 1 (115‰), 2 (115‰), 3 (107‰), 4 (101‰), 5 (93‰), 6 (93‰).

Plant material was obtained by vegetatively propagating the selected genotypes from the stock populations, which had been maintained under non-saline conditions in a temperature controlled glasshouse for six to eight vegetative generations prior to the beginning of this experiment as described in Part I.

Experimental design

The experimental design for each species was a factorial randomized block design of six replicates, or blocks, that blocked on bench variation within the growth chamber. The three species experiments were run sequentially, not simultaneously. For each species the following 6 x 2 x 2 factorial arrangement of main effects was tested: population effect (six populations ranging from high to low salinity tolerance), treatment effect (sublethal salinity level versus control at non-saline conditions), harvest effect (early harvest one week after exposure to sublethal salinity level and late harvest five weeks after exposure to sublethal salinity level). This experimental design yielded a total of 144 experimental units for each species experiment.

All experiments were conducted in a temperature-controlled EGC walk-in growth chamber set to 16 hr daylength at 30 C and an 8 hr dark period at 24 C as described in Part I. Depending on the size of the plants, two to four young stems were planted per pot and placed inside larger reservoir pots that contained the treatment bathing solution at the desired salinity. Initial plant wet weight was recorded and tested for statistical significance for use as a covariable with harvest total plant biomass.

Each experiment consisted of two harvests: an early harvest one week after exposure to the designated sublethal salinity level and a late harvest after four additional weeks (five weeks total exposure to the sublethal salinity level). Additional information on variables measured in each harvest is provided in Part I.

Salinity regime

The sublethal salinity level varied depending on the species as follows: Panicum hemitomon, 4‰; Spartina patens, 20‰; and Spartina alterniflora, 30‰. All experimental units were initially maintained in a treatment bathing solution of half-strength Hoagland's nutrient solution (Hoagland and Arnon 1950) at 0‰ salinity for a two week period following transplanting. Salinity was then increased stepwise over a relatively short time interval until the targeted sublethal salinity level was reached using a commercial sea salts mix (Instant Ocean®; Aquarium Systems, Mentor, Ohio) in half-strength Hoagland's as described in Part I. Controls were kept at 0‰ salinity in half-strength Hoagland's except for the Spartina alterniflora controls, which were provided with 1‰ sea salt in half-strength Hoagland's. All bathing solutions were drained and replaced at weekly intervals and salinities were checked twice weekly throughout each experiment.

Harvest

At harvest, aboveground tissue was lightly rinsed with distilled/deionized water to remove any encrusted salts on the leaf surfaces. Green leaf tissue was clipped from the upper leaves of each stem, frozen in liquid N_2, and lyophilized (freeze-dried) for analysis of leaf cations and osmotic compounds (reported in Chapter 6, Part III of this series). Upon removal from the lyophilizer, the lyophilized green leaf tissue was weighed, recorded, and then stored in a dessicator under vacuum. Dry weight of the lyophilized leaf tissue was subsequently added to the live aboveground biomass partition of each respective sample. Soil from the belowground portion of each experimental unit was carefully rinsed with tap water over a 2 mm mesh screen. The attached aboveground stems and leaves were partitioned into live and dead components. Belowground biomass was partitioned into root and rhizome components. Samples were oven dried at 65 C until constant weight was achieved (four days) and dry weight determined.

Data analysis

Data was analyzed as a factorial randomized block design. Analysis of variance (ANOVA) was used to test for significant main effects and interactions using SAS (SAS 1989; Steel and Torrie 1980). A significance (alpha) level of 0.05 was used for all analyses unless otherwise stated. Single degree of freedom contrasts were used to make a priori comparisons between populations of different degrees of salt tolerance. All data were tested for meeting the assumptions of normality and homogeneity of variance by using a combination of the Shapiro-Wilk test statistic for tests of normality and the Bartlett test for tests of homogeneity of variance (SAS 1989). Data that did not meet these assumptions were transformed until assumptions were met.

RESULTS

Panicum hemitomon

Plant wet weight at the time of planting did show significant population differences despite efforts to minimize this effect and, therefore, was used as a covariable in the analysis of total plant biomass from the early and late harvests. Covariable-adjusted total plant biomass displayed highly significant harvest, treatment and population main effects, as well as a significant harvest x treatment interaction. Despite the significant interaction of harvest with treatment, all populations subjected to the salinity treatment tended to have lower covariable-adjusted total plant biomass than their respective controls, with this difference being much greater in the late harvest (Figure 5.1). In the early harvest there were no significant differences between highly salt-tolerant populations (populations 1 and 2) and poorly salt-tolerant populations (populations 5 and 6) in contrasts of covariable-adjusted total plant biomass within treatments or controls. Similarly, contrasts within treatments of population 1 (15.0 g per pot) versus population 6 (12.7 g per pot) failed to show significant differences in covariable-adjusted total plant biomass in the early harvest. However, late harvest contrasts between the highly salt-tolerant and poorly salt-tolerant populations did show

significantly greater covariable-adjusted total plant biomass in the highly salt-tolerant populations within treatments, as well as within controls (Figure 5.1). In the late harvest treatments, populations 1 and 2 had significantly greater covariable-adjusted total plant biomass (20.3 and 15.9 g per pot, respectively) than populations 5 and 6 (8.4 and 9.1 g per pot, respectively; Figure 5.1).

Live, dead, and total aboveground biomass also displayed significant harvest, treatment and population effects and a harvest x treatment interaction. Contrasts of highly salt-tolerant populations versus poorly salt-tolerant populations revealed significant differences in these aboveground components within treatments, but not within controls (Figure 5.2). Within treatments of the early harvest, total aboveground biomass for populations 1 and 2 was 11.7 and 11.0 g per pot, respectively, compared to values of 8.2 and 10.3 g per pot for populations 5 and 6, respectively. Late harvest differences were more dramatic with populations 1 and 2 having respective total aboveground biomass values of 13.7 and 10.6 g per pot compared to values for populations 5 and 6 of 3.9 and 6.2 g per pot, respectively (Figure 5.2).

The proportion of dead aboveground biomass (dead aboveground biomass divided by total aboveground biomass) showed significant main effects of harvest, treatment, and population and also a significant treatment x population interaction as evidenced by the greater proportion of dead biomass in the poorly salt-tolerant populations (Figure 5.3). Contrasts within treatments between highly salt-tolerant and poorly salt-tolerant populations showed that in both the early and late harvests, the poorly salt-tolerant populations had a significantly greater proportion of dead aboveground biomass than the highly salt-tolerant populations, whereas no significant differences were detected within the controls of either harvest (Figure 5.3).

Total belowground biomass, root biomass, and rhizome biomass all showed significant main effects and significant interactions of harvest x treatment and harvest x population. Belowground components showed similar trends with root and rhizome

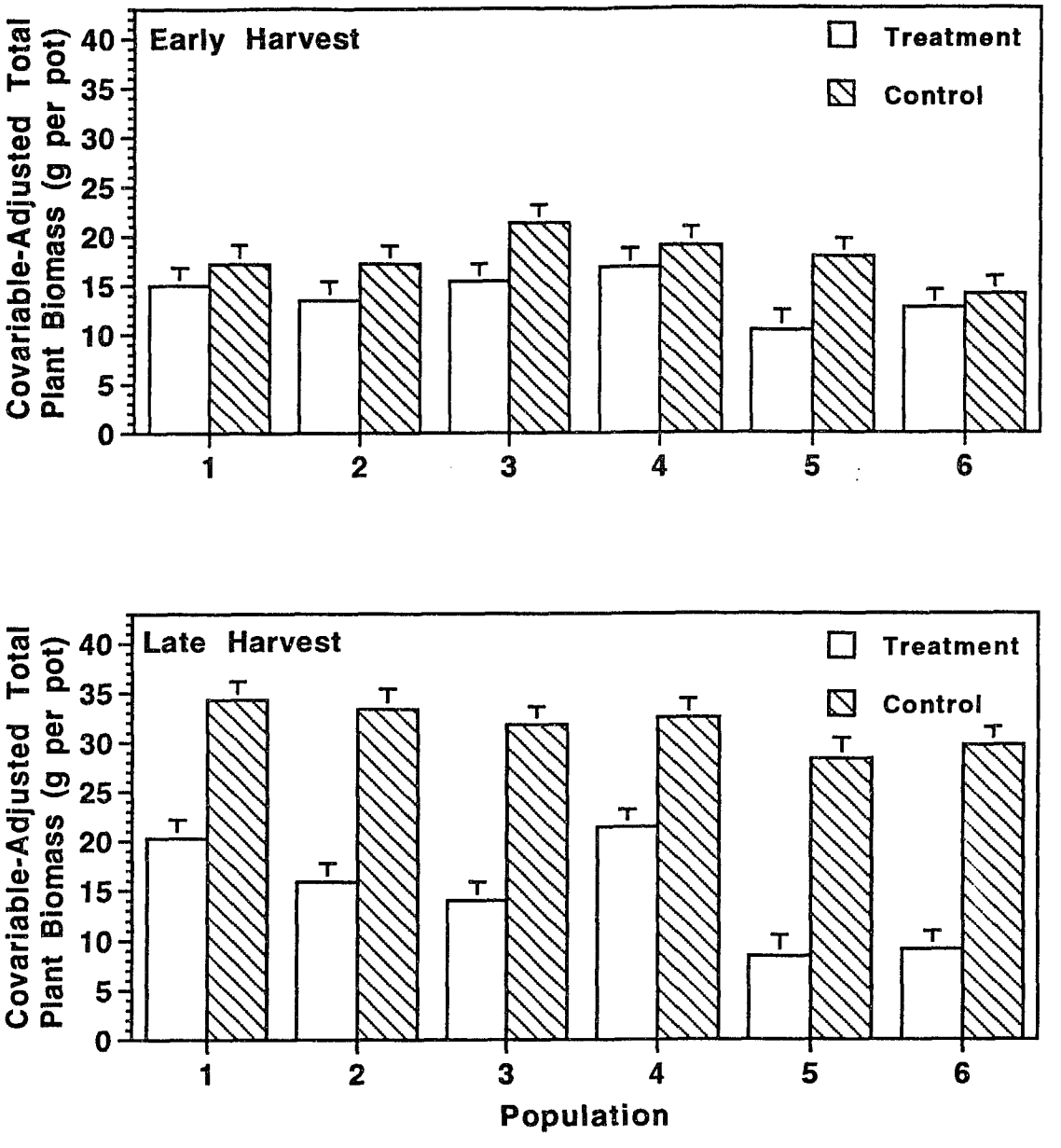

Figure 5.1. Mean (± std err) covariable-adjusted total plant biomass (g per pot) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Panicum hemitomon when subjected to a sublethal salinity excursion of 4‰ for one week (early harvest) and five weeks (late harvest); n=6, $LSD_{0.05}$=5.26, MSE=20.49.

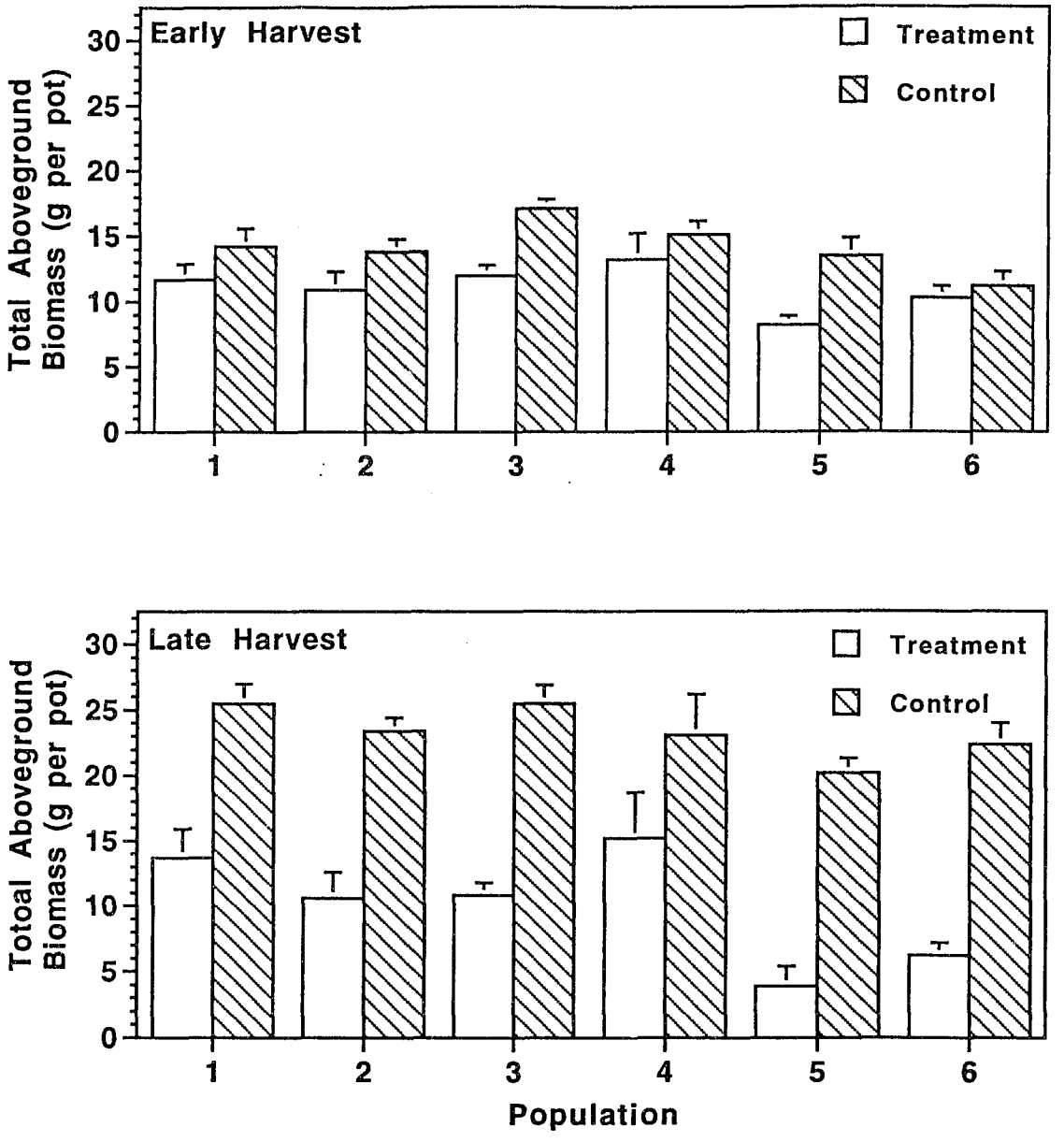

Figure 5.2. Mean (± std err) total aboveground biomass (g per pot) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Panicum hemitomon when subjected to a sublethal salinity excursion of 4‰ for one week (early harvest) and five weeks (late harvest); n=6, $LSD_{0.05}$=4.23, MSE=13.32.

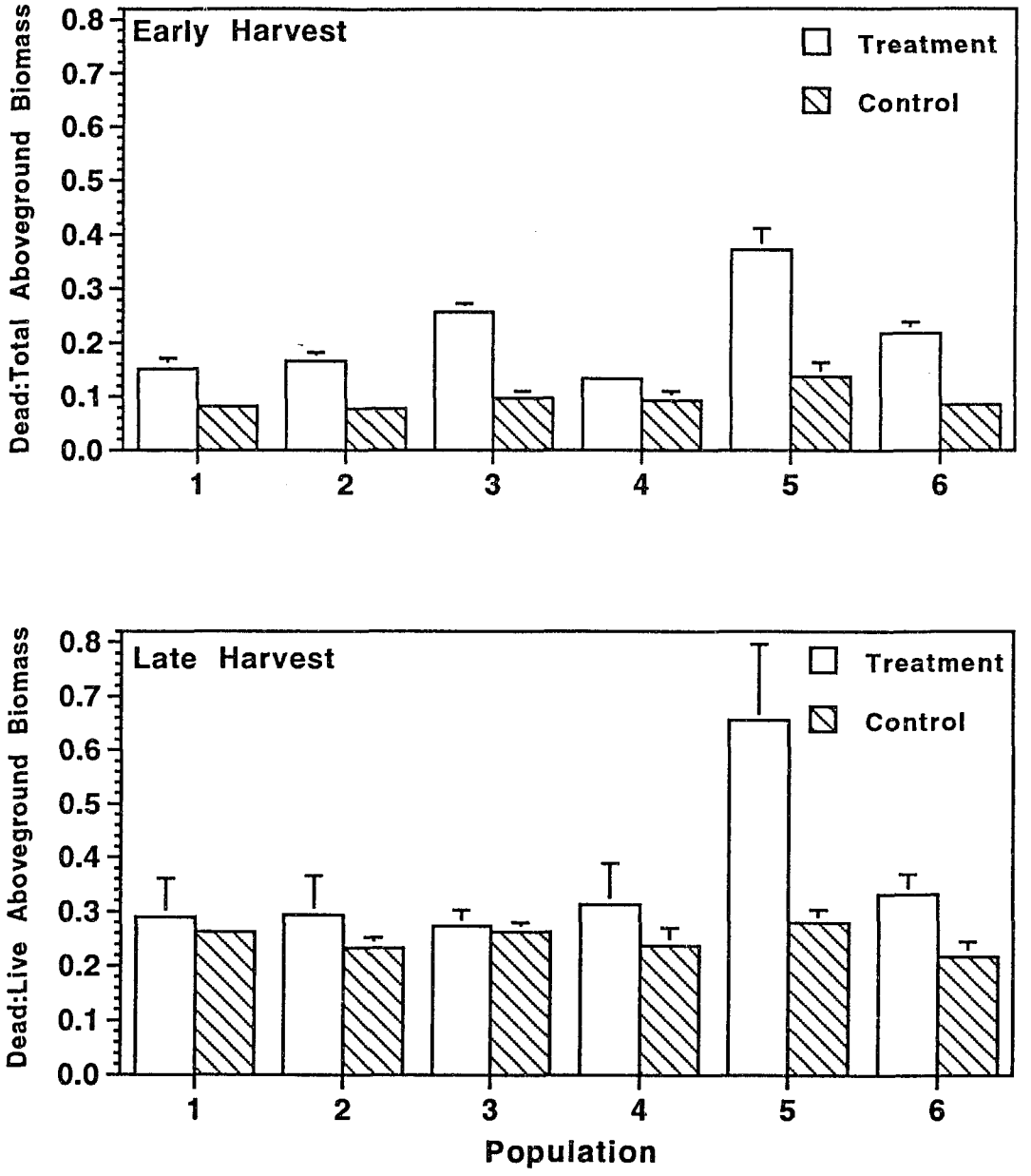

Figure 5.3. Mean (± std err) dead:total aboveground biomass ratio (proportion of dead aboveground biomass) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Panicum hemitomon when subjected to a sublethal salinity excursion of 4‰ for one week (early harvest) and five weeks (late harvest); n=6, $LSD_{0.05}$=0.117, MSE=0.010.

biomass nearly evenly partitioned. The interactions of harvest x treatment and harvest x population can be seen in Figure 5.4 and can be attributed to the lack of a treatment effect in the early harvest and greater belowground biomass within treatments of the highly salt-tolerant populations in the late harvest. Contrasts within late harvest treatments and controls showed that the poorly salt-tolerant populations had significantly less total belowground biomass than the highly salt-tolerant populations, and that the difference within treatments was much greater than that observed within controls (Figure 5.4). Late harvest treatment values of total belowground biomass for populations 1 and 2 (highly salt tolerant) were 4.9 and 4.2 g per pot, respectively, compared to 1.9 and 2.6 g per pot for populations 5 and 6 (poorly salt tolerant), respectively (Figure 5.4). Root biomass within treatments of highly salt-tolerant populations was significantly greater than that of poorly salt-tolerant populations in both harvests (Figure 5.5). Root biomass within controls of the poorly salt-tolerant populations was significantly less than that of the highly salt-tolerant controls only in the late harvest (Figure 5.5)

Root-to-shoot ratios showed significant main effects and a significant harvest x treatment interaction (Figure 5.6). Controls did not show a significant difference between highly salt-tolerant and poorly salt-tolerant populations in either harvest. In the late harvest treatments, the poorly salt-tolerant populations had significantly greater root-to-shoot ratios than the highly salt-tolerant populations (Figure 5.6). However, this difference appears to be primarily due to the large root-to-shoot ratio of population 5, especially in light of the fact that root-to-shoot ratio of population 1 was not significantly different than that of population 6.

Spartina patens

Plant wet weight at the time of planting did not show significant population differences and, therefore, was not used as a covariable for total plant biomass at harvest. All main effects and interactions were significant for total plant biomass (Figure 5.7). In the early harvest there was not a significant treatment effect nor a significant

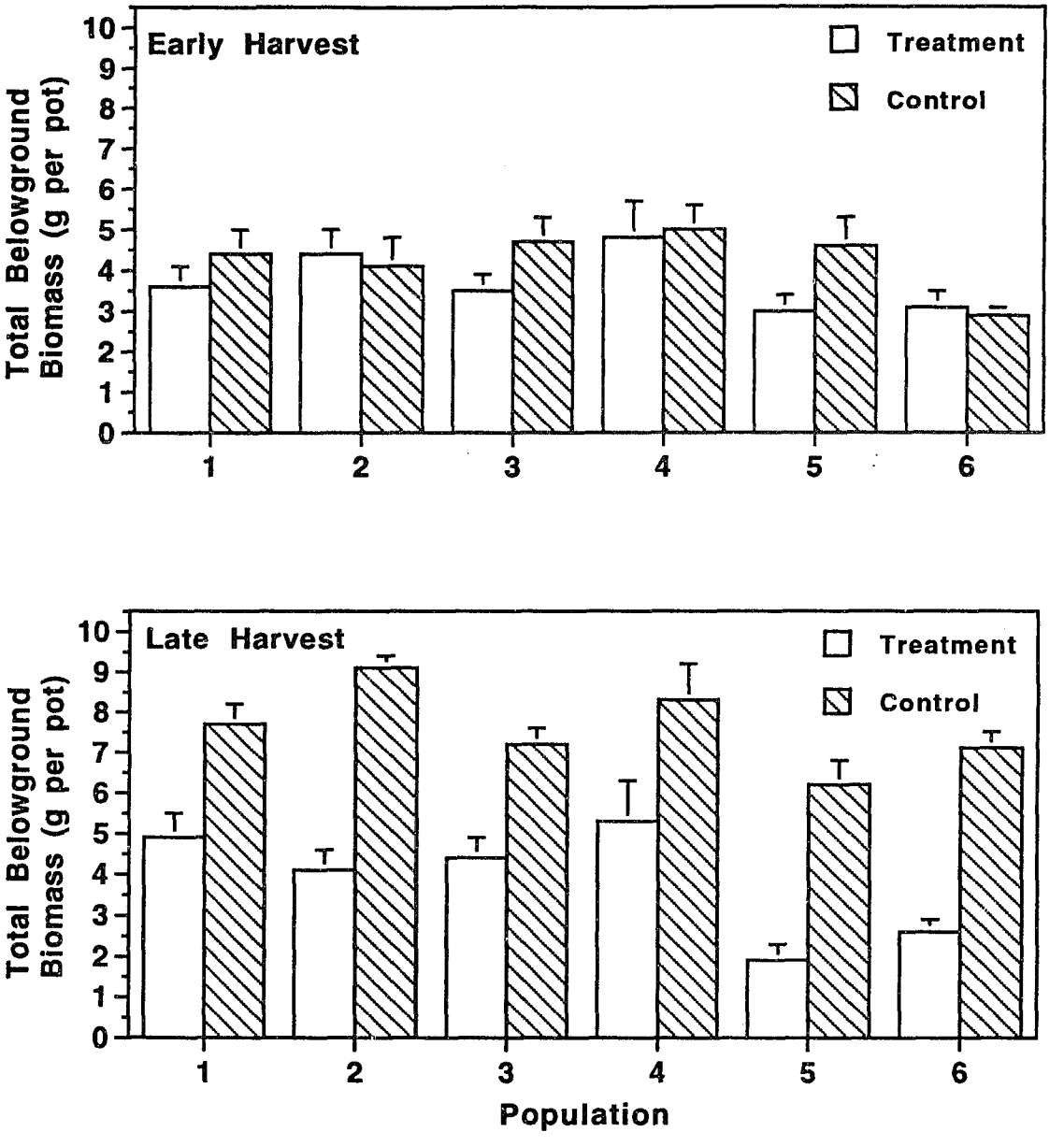

Figure 5.4. Mean (± std err) total belowground biomass (root plus rhizome; g per pot) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Panicum hemitomon when subjected to a sublethal salinity excursion of 4‰ for one week (early harvest) and five weeks (late harvest); n=6, $LSD_{0.05}$=1.46, MSE=1.62.

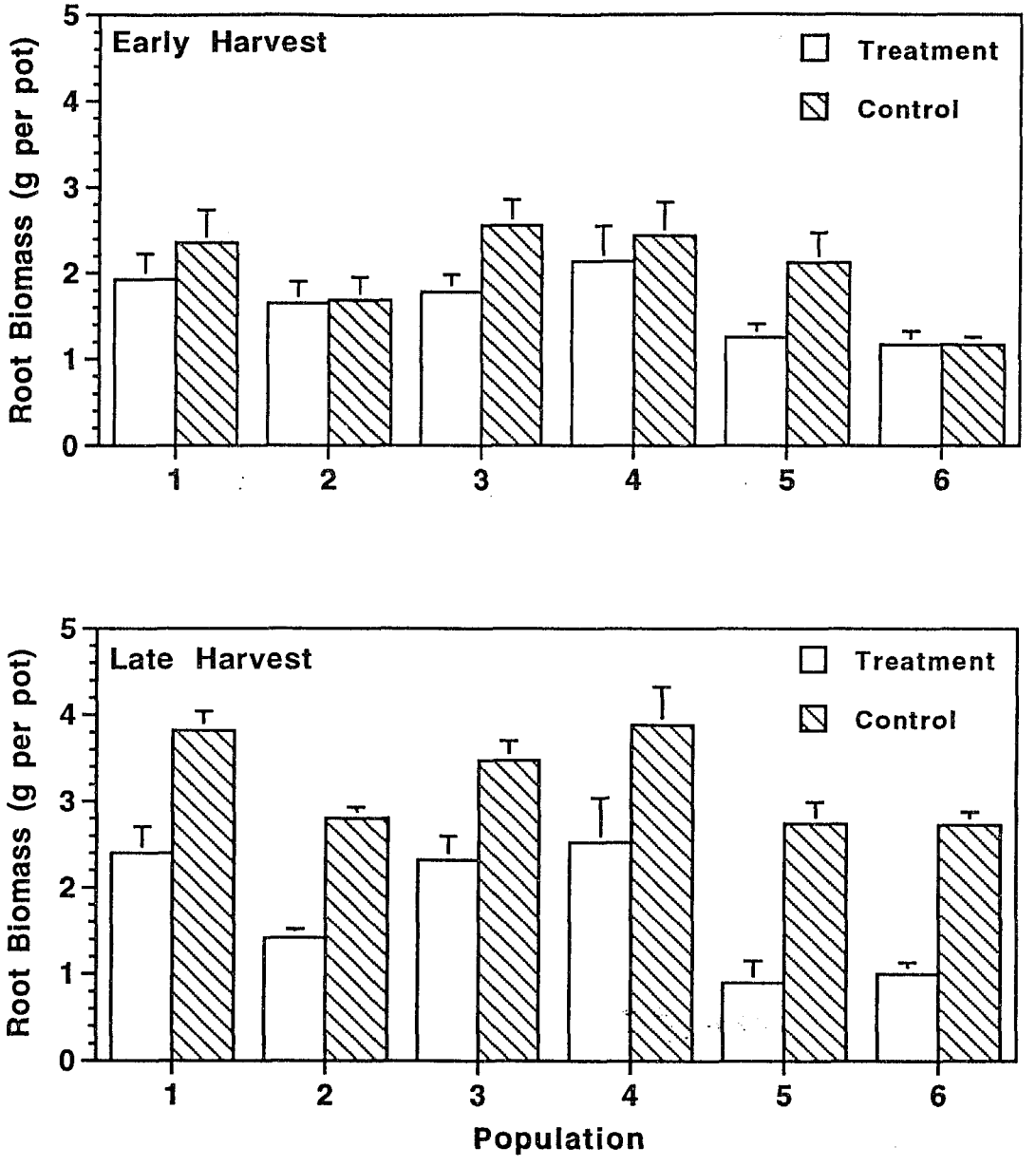

Figure 5.5. Mean (± std err) root biomass (g per pot) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Panicum hemitomon when subjected to a sublethal salinity excursion of 4‰ for one week (early harvest) and five weeks (late harvest); n=6, $LSD_{0.05}$=0.712, MSE=0.384.

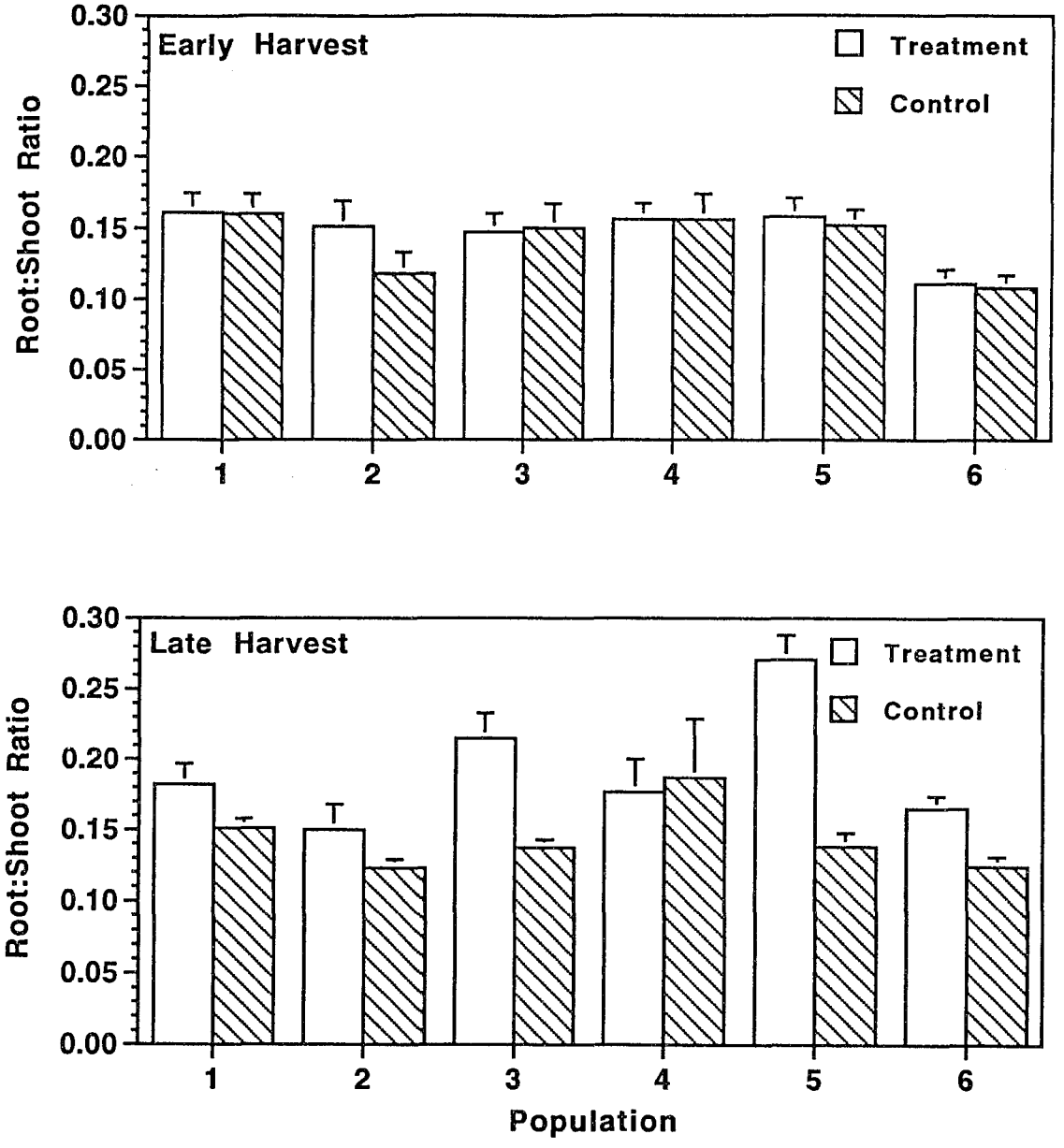

Figure 5.6. Mean (± std err) root:shoot ratio (root biomass/total aboveground biomass) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Panicum hemitomon when subjected to a sublethal salinity excursion of 4‰ for one week (early harvest) and five weeks (late harvest); n=6, $LSD_{0.05}$=0.045, MSE=0.001.

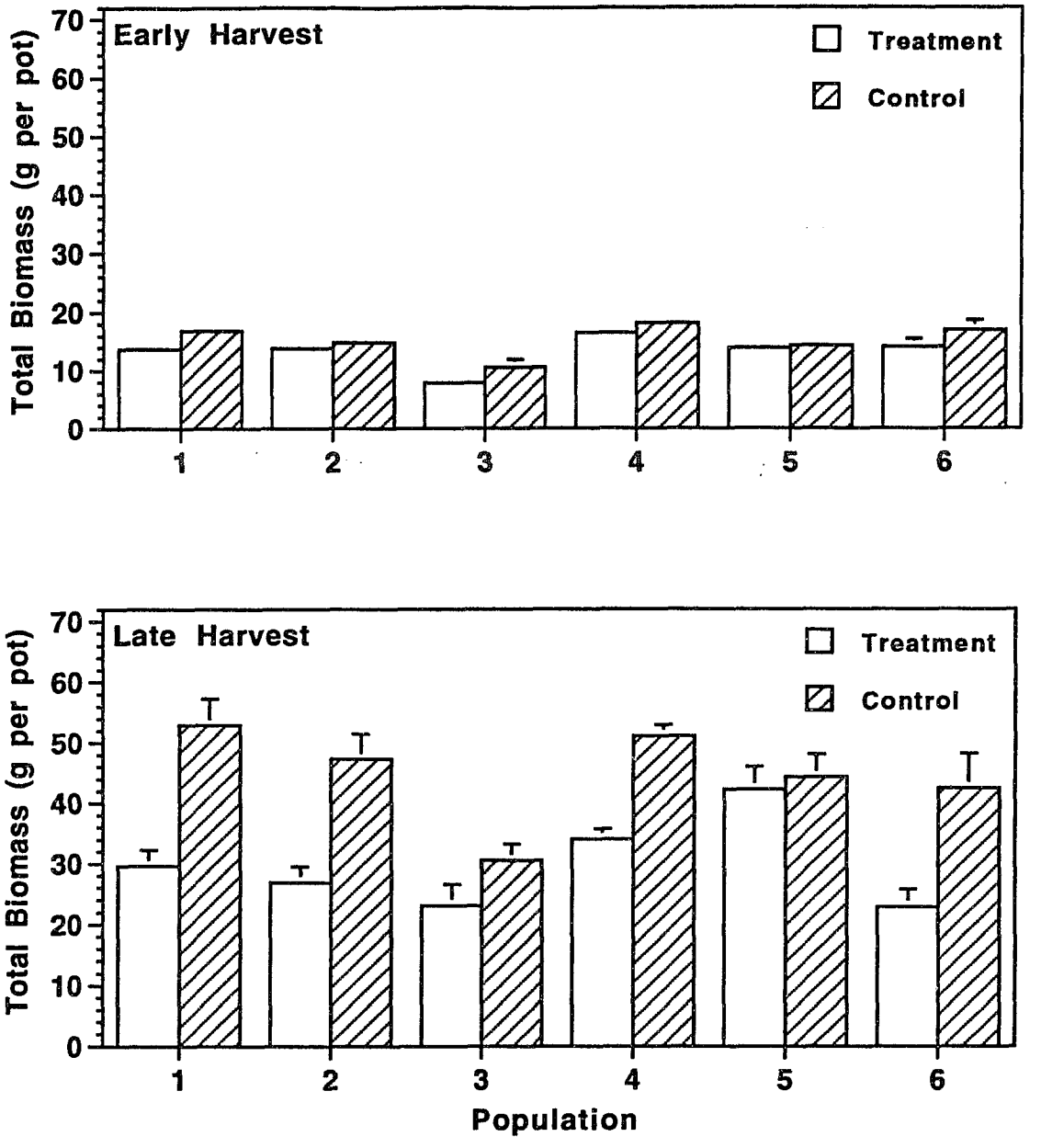

Figure 5.7. Mean (± std err) total plant biomass (g per pot) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Spartina patens when subjected to a sublethal salinity excursion of 20‰ for one week (early harvest) and five weeks (late harvest); n=6, $LSD_{0.05}$=6.52, MSE=32.50.

difference between populations within controls or within treatments in total plant biomass. In the late harvest, contrasts within treatments showed that population 1 had significantly greater biomass (29.6 g per pot) than population 6 (22.9 g per pot), but a contrast of highly salt-tolerant populations (1 and 2) versus poorly salt-tolerant populations (5 and 6) was not significant, apparently because of the relatively high treatment biomass of population 5 (Figure 5.7). Within late harvest controls the highly salt-tolerant populations did show significantly greater total plant biomass than the poorly salt-tolerant populations (Figure 5.7).

Aboveground live and aboveground total biomass showed significant harvest, treatment and population main effects and significant harvest x treatment and harvest x population interactions (Figure 5.8). In the early harvest no significant differences were detected between highly salt-tolerant and poorly salt-tolerant populations within treatments or controls (Figure 5.8). In the late harvest, population 1 (high salinity tolerance) had greater total aboveground biomass (25.0 and 43.1 g per pot for the treatment and control, respectively) than population 6 (low salinity tolerance; 19.3 and 34.6 g per pot for the treatment and control, respectively; Figure 5.8). However, contrasts that tested high salinity tolerance populations (populations 1 and 2) against low salinity tolerance populations (populations 5 and 6) were not significant.

The proportion of dead aboveground biomass showed significant harvest and treatment effects, but not a significant population effect. Inspection of Figure 5.9 shows that these treatment differences were relatively small with salinity treatments averaging 5% and 8% dead aboveground biomass for early and late harvests, respectively, compared to respective control values of 3% and 7% (Figure 5.9).

Root, rhizome, and total belowground biomass all showed significant main effects and interactions (Figures 5.10 and 5.11). Interestingly, within the late harvest treatments the root biomass of population 5 (8.3 g per pot) was significantly greater than its control and all other treatment combinations (Figure 5.11). Otherwise, root and

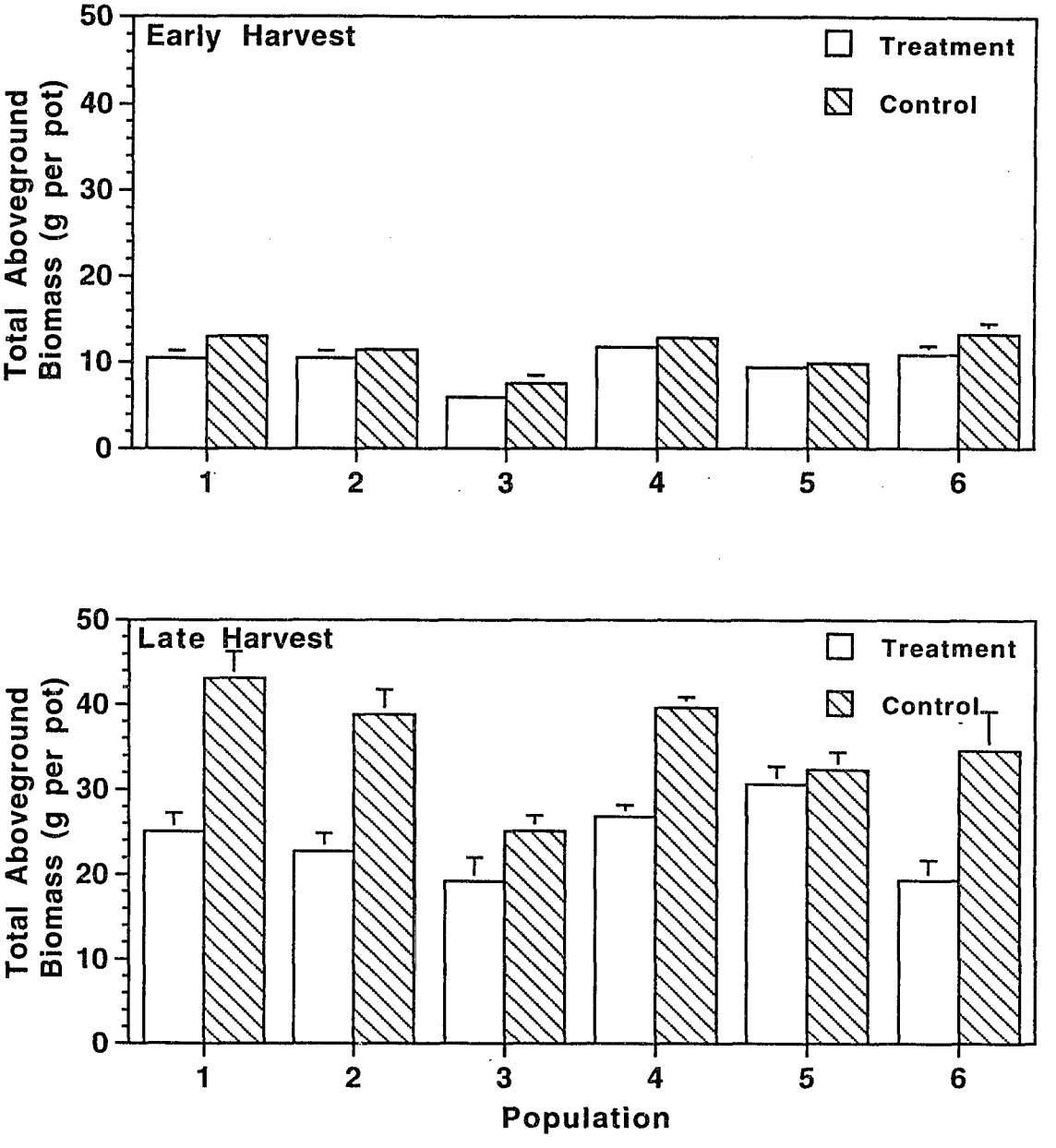

Figure 5.8. Mean (± std err) total aboveground biomass (g per pot) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Spartina patens when subjected to a sublethal salinity excursion of 20‰ for one week (early harvest) and five weeks (late harvest); n=6, $LSD_{0.05}$=5.01, MSE=19.19.

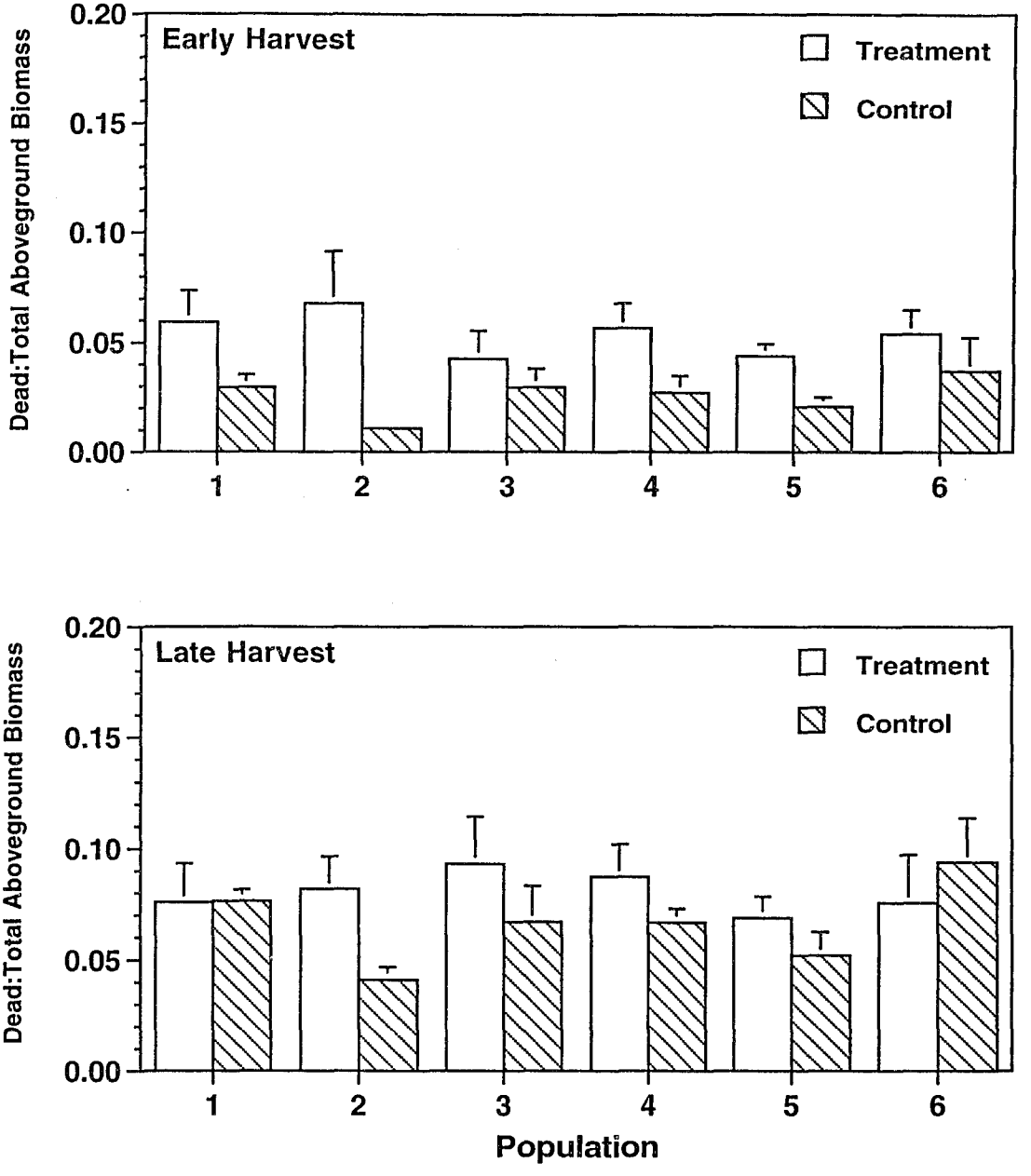

Figure 5.9. Mean (± std err) dead:total aboveground biomass ratio (proportion of dead aboveground biomass) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Spartina patens when subjected to a sublethal salinity excursion of 20‰ for one week (early harvest) and five weeks (late harvest); n=6, $LSD_{0.05}$=0.030, MSE=0.0007.

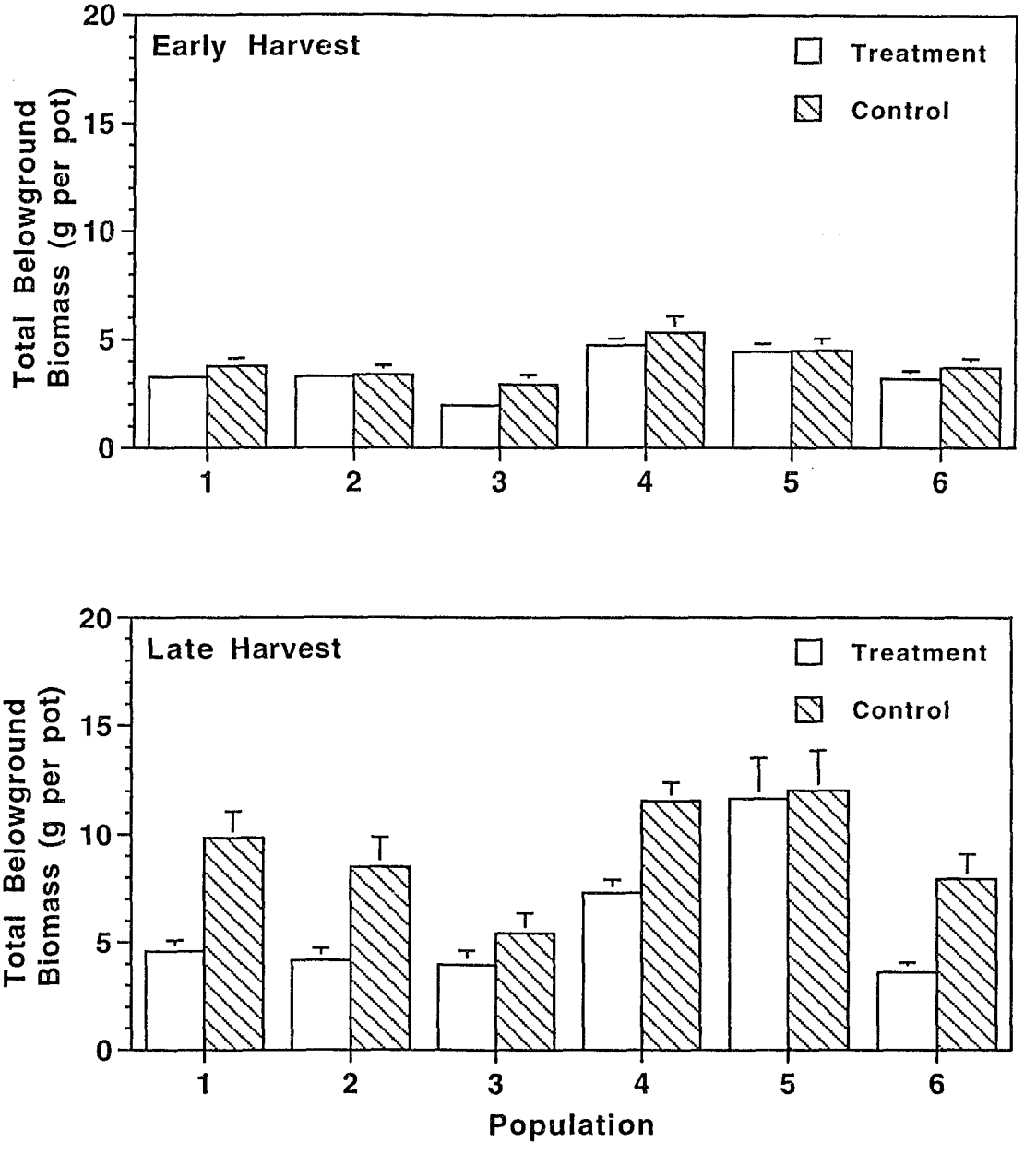

Figure 5.10. Mean (± std err) total belowground biomass (root plus rhizome; g per pot) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Spartina patens when subjected to a sublethal salinity excursion of 20‰ for one week (early harvest) and five weeks (late harvest); n=6, $LSD_{0.05}$=2.01, MSE=3.08.

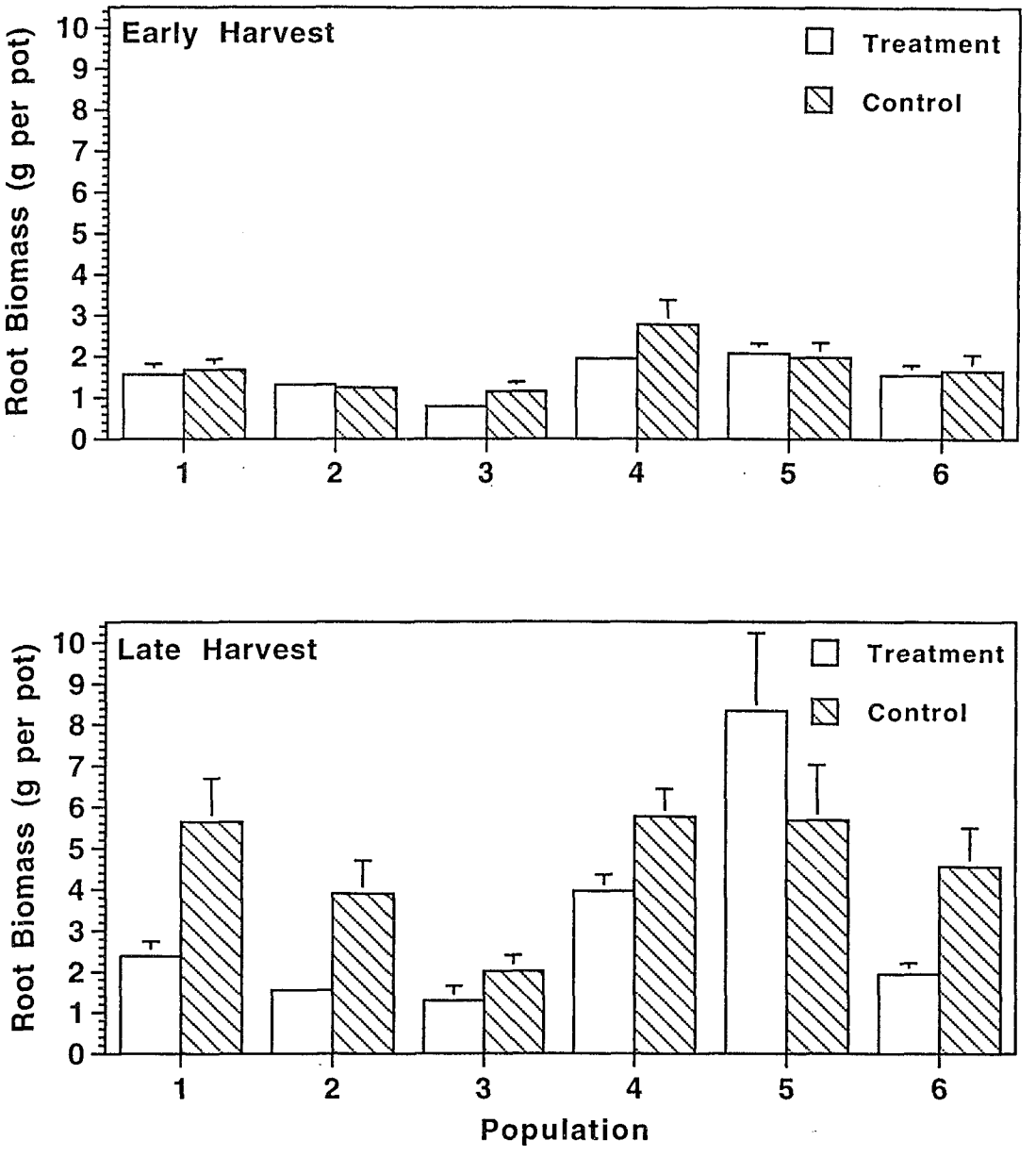

Figure 5.11. Mean (± std err) root biomass (g per pot) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Spartina patens when subjected to a sublethal salinity excursion of 20‰ for one week (early harvest) and five weeks (late harvest); n=6, $LSD_{0.05}$=1.67, MSE=2.14.

rhizome biomass were generally fairly evenly distributed. Contrasts within the salinity treatments for populations of high salinity tolerance (1 and 2) versus populations of low salinity tolerance (5 and 6) showed no significant differences for roots, rhizomes or total belowground biomass in the early harvest, but did show significant differences for roots and total belowground in the late harvest, apparently due to the high root biomass of population 5 (Figures 5.10 and 5.11). Contrasts within treatments of population 1 versus population 6 did not yield significant differences for roots, rhizomes, or total belowground biomass for either the early or late harvests. Within controls, there were no significant differences between highly salt-tolerant and poorly salt-tolerant populations in terms of root, rhizome, or total belowground biomass in either harvest.

Root-to-shoot ratios did not display a significant treatment effect, although there was a significant population effect and significant harvest x treatment and harvest x population interactions (Figure 5.12). In general, root-to-shoot ratios were larger in the early harvest than in the late harvest, particularly for population 5 (Figure 5.12). Contrasts within treatments and controls between highly salt-tolerant populations and poorly salt-tolerant populations were significant only for the early harvest, with the poorly salt-tolerant populations having the larger root-to-shoot ratios (Figure 5.12).

Spartina alterniflora

Initial plant wet weight at the time of planting did show significant population differences and, therefore, was used as a covariable in analyses of total plant biomass in both early and late harvests. Covariable-adjusted total plant biomass, live aboveground biomass, and total aboveground biomass all had significant harvest, treatment, and population main effects, as well as significant harvest x treatment and harvest x population interactions. Late harvest biomass values were greater than early harvest values within the controls, whereas treatment values did not show much of an increase between early and late harvests (Figures 5.13 and 5.14). Contrasts within treatments revealed no significant differences between highly salt-tolerant and poorly salt-tolerant

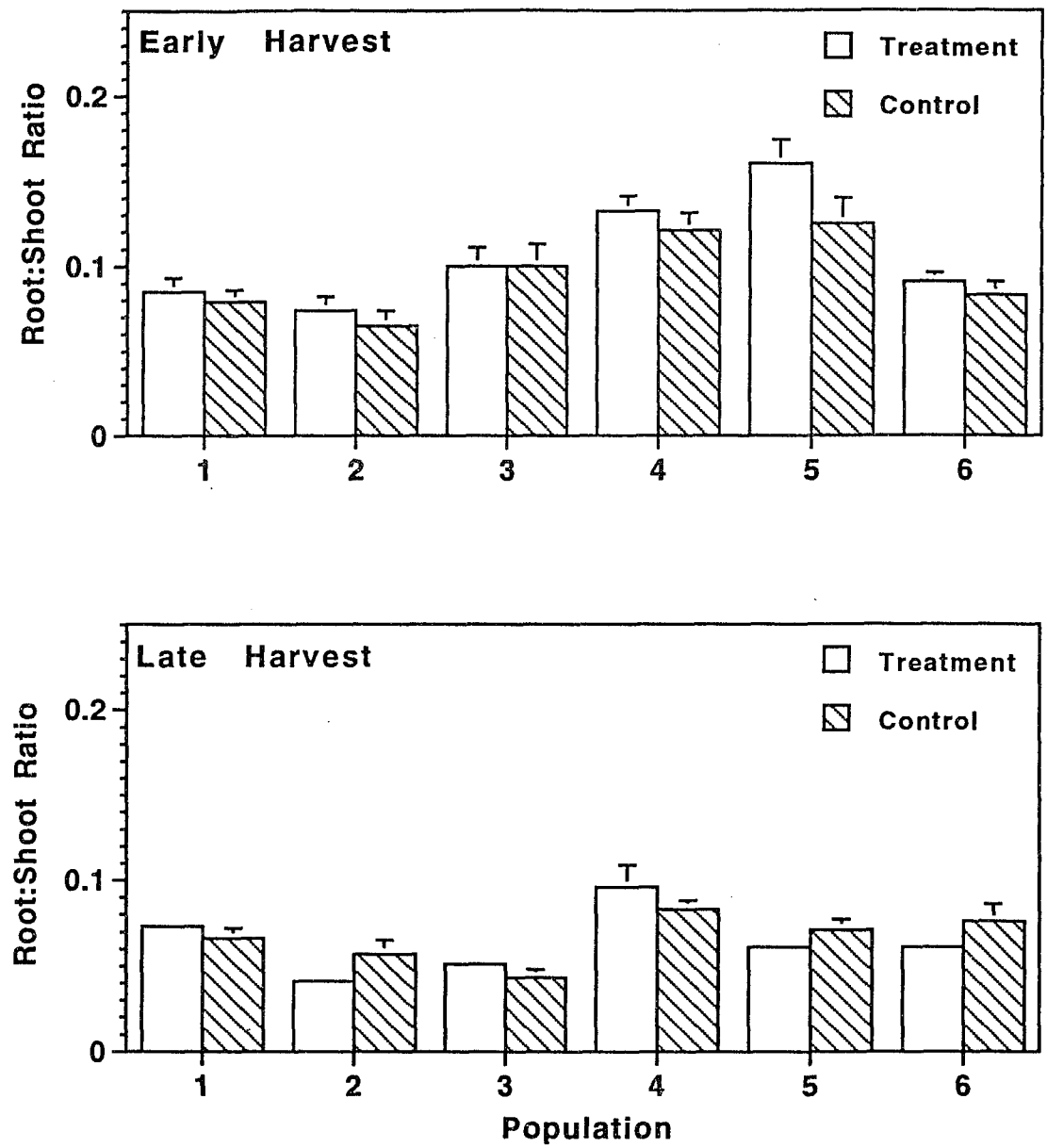

Figure 5.12. Mean (± std err) root:shoot ratio (root biomass/total aboveground biomass) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Spartina patens when subjected to a sublethal salinity excursion of 20‰ for one week (early harvest) and five weeks (late harvest); n=6, $LSD_{0.05}$=0.021, MSE=0.0003.

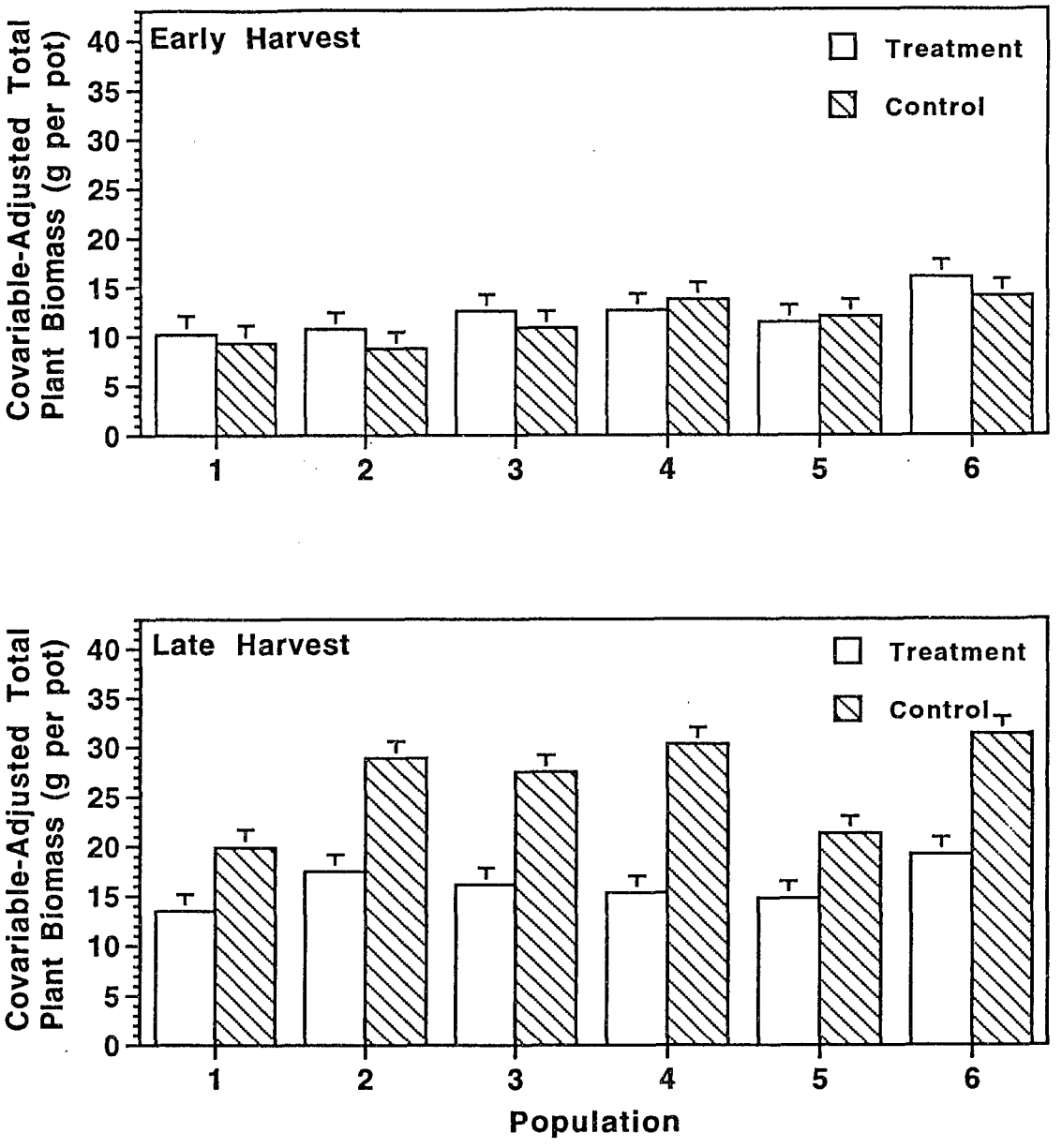

Figure 5.13. Mean (± std err) covariable-adjusted total plant biomass (g per pot) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Spartina alterniflora when subjected to a sublethal salinity excursion of 30‰ for one week (early harvest) and five weeks (late harvest); n=6, $LSD_{0.05}$=5.08, MSE=19.39.

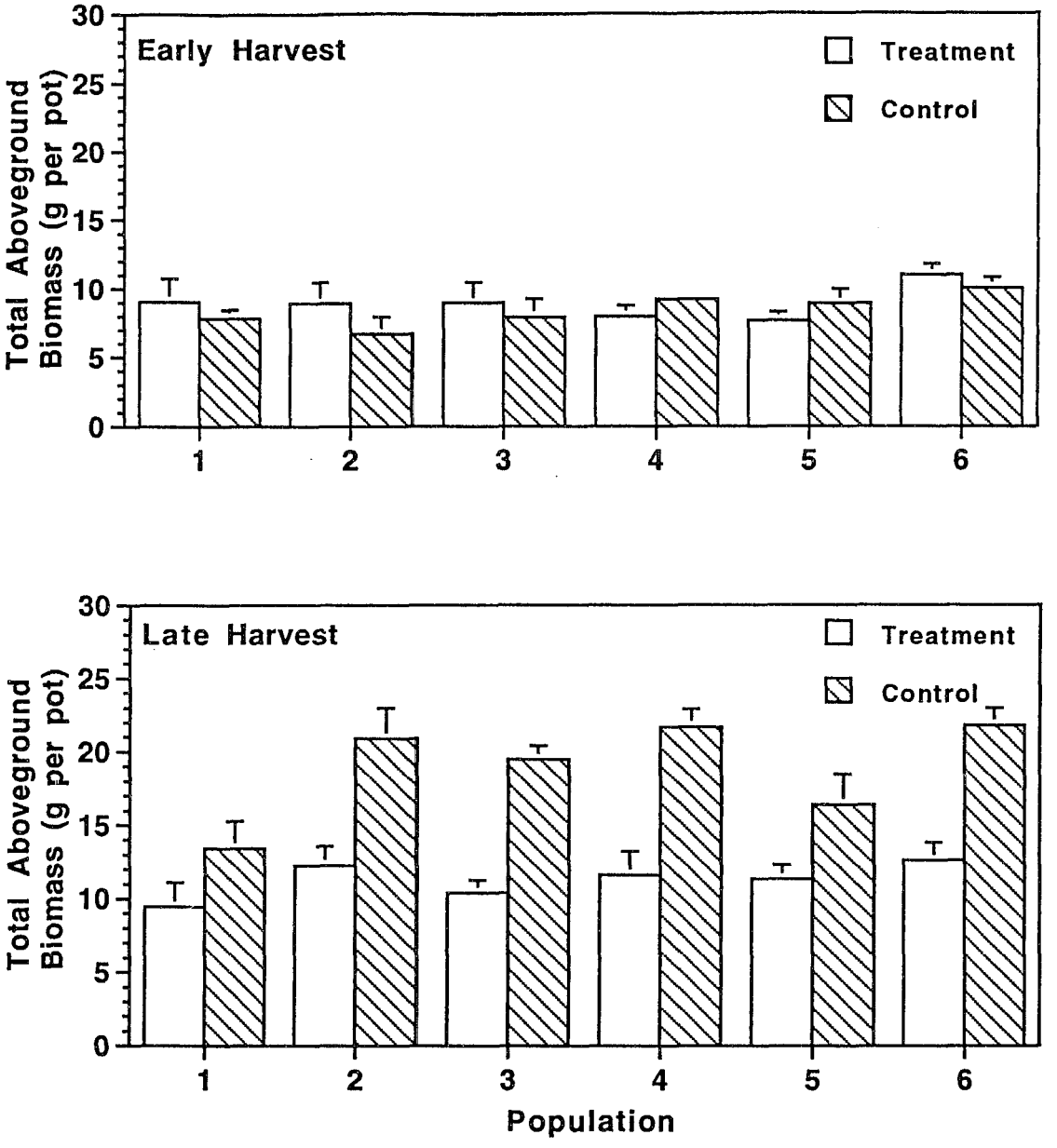

Figure 5.14. Mean (± std err) total aboveground biomass (g per pot) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Spartina alterniflora when subjected to a sublethal salinity excursion of 30‰ for one week (early harvest) and five weeks (late harvest); n=6, $LSD_{0.05}$=3.61, MSE=9.82.

populations in terms of covariable-adjusted total plant biomass for either harvest (Figure 5.13), although contrasts within controls showed that the early harvest poorly salt-tolerant populations had greater covariable-adjusted total plant biomass than the highly salt-tolerant populations. Covariable-adjusted total plant biomass ranged from 13 to 19 g per pot for late harvest treatments compared to 19 to 31 g per pot for late harvest controls (Figure 5.13). Contrasts within treatments and controls did not reveal any significant differences between highly salt-tolerant populations and poorly salt tolerant populations in live or total aboveground biomass (Figure 5.14)

The proportion of dead aboveground biomass (dead aboveground/total aboveground) displayed significant treatment and population effects, as well as a significant treatment x population interaction (Figure 5.15). Contrasts revealed no differences within controls, whereas contrasts within treatments showed that poorly salt-tolerant populations had a significantly greater proportion of dead aboveground biomass than the highly salt-tolerant populations in both harvests (Figure 5.15). Highly salt-tolerant populations had proportions of dead aboveground biomass that averaged about two-thirds those of the poorly salt-tolerant populations (Figure 5.15).

Root and total belowground biomass both showed significant main effects and significant harvest x treatment and harvest x population interactions. As with the other biomass parameters, the controls showed a greater increase between early and late harvests than the treatments (Figures 5.16 and 5.17). Interestingly, most of the early harvest treatments produced as much, if not more root and total belowground biomass than their respective controls (Figures 5.16 and 5.17). Roots and total belowground biomass showed a trend of increasing biomass in the less salt-tolerant populations, particularly within the treatments (Figures 5.16 and 5.17). Within both controls and treatments, contrasts of the highly salt-tolerant populations versus the poorly salt-tolerant populations showed that the highly salt-tolerant populations had significantly less root and total belowground biomass than the poorly salt-tolerant populations (Figures 5.16

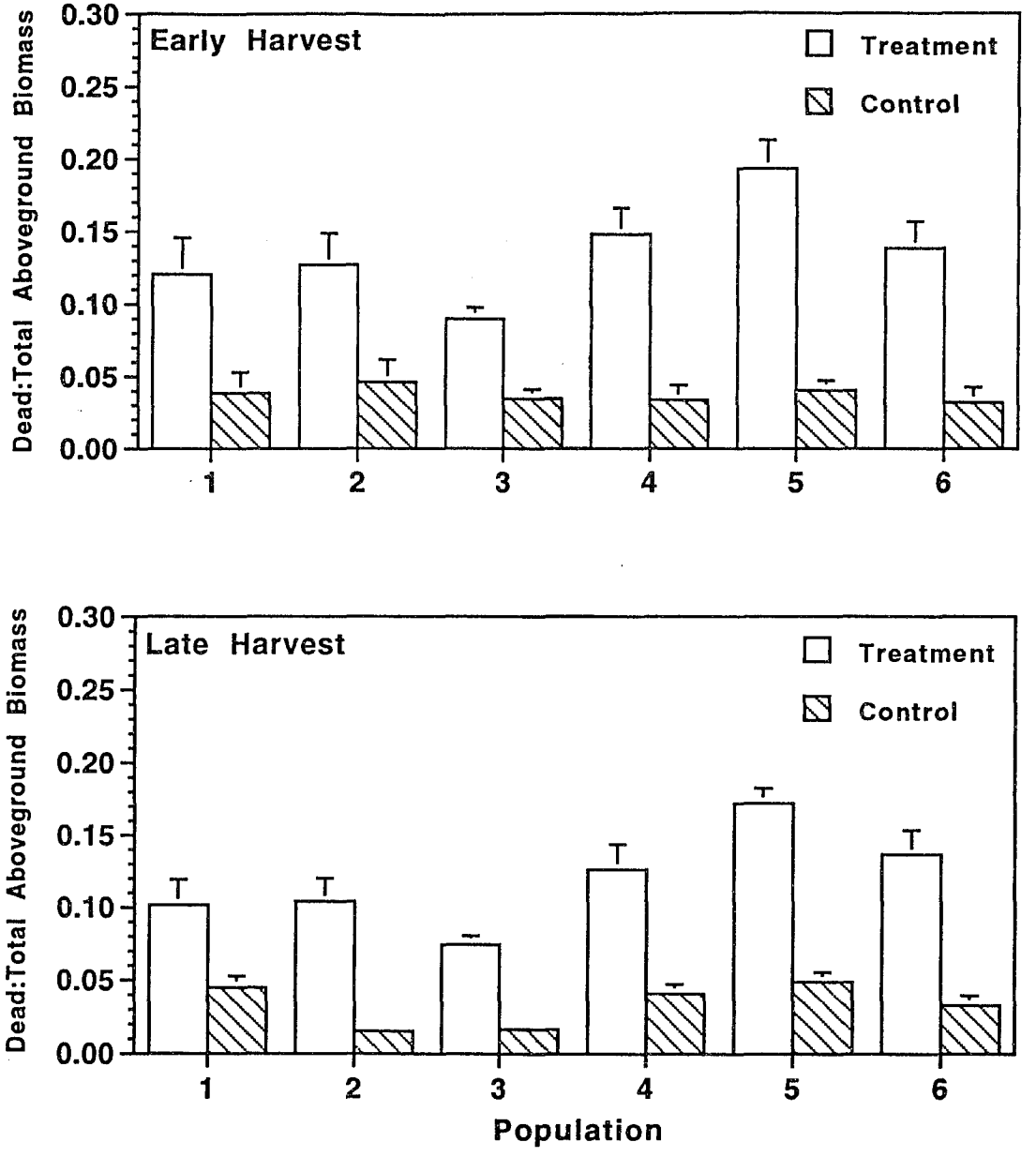

Figure 5.15. Mean (± std err) dead:total aboveground biomass ratio (proportion of dead aboveground biomass) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Spartina alterniflora when subjected to a sublethal salinity excursion of 30‰ for one week (early harvest) and five weeks (late harvest); n=6, $LSD_{0.05}$=0.038, MSE=0.0011.

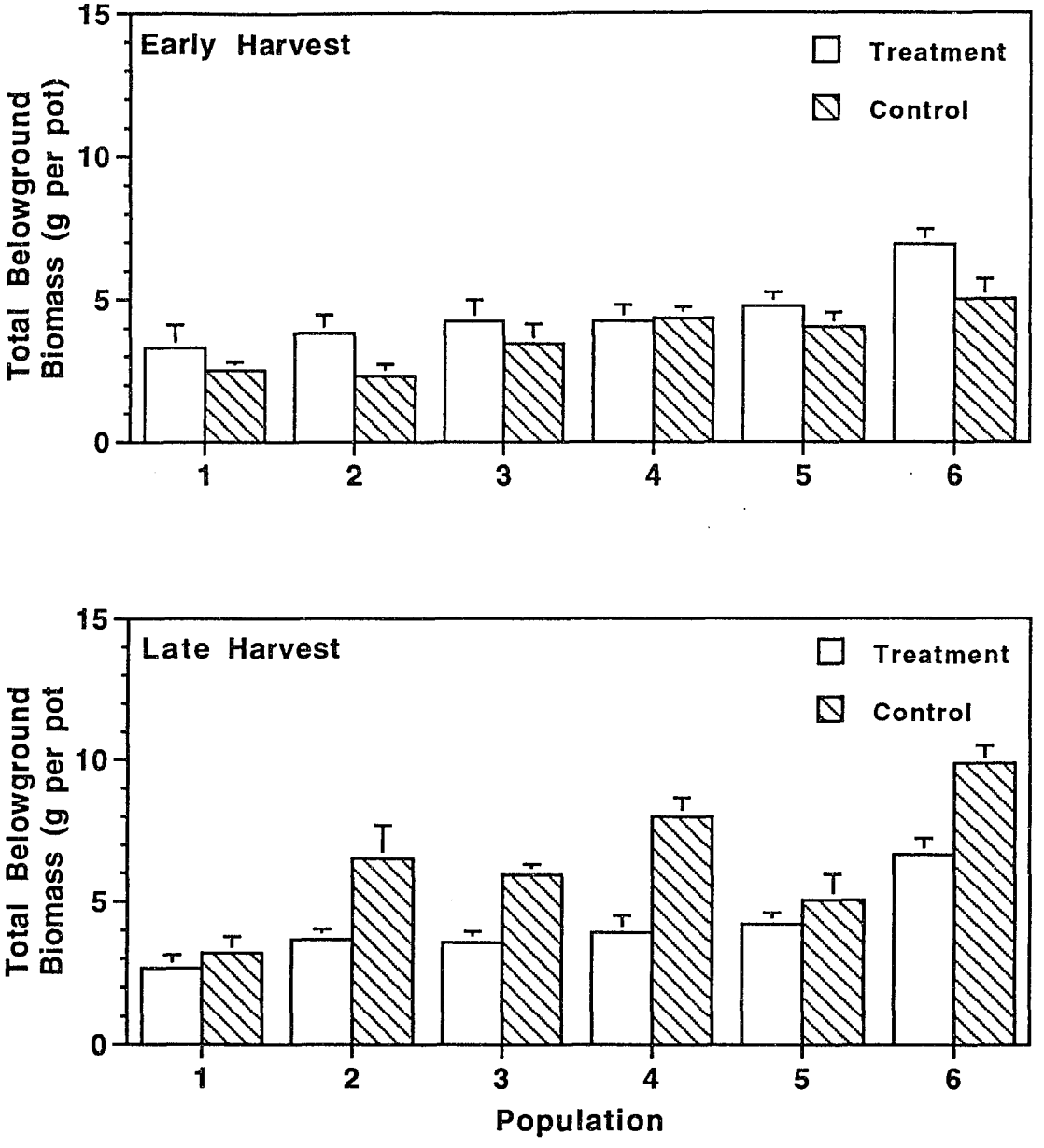

Figure 5.16. Mean (± std err) total belowground biomass (root plus rhizome; g per pot) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Spartina alterniflora when subjected to a sublethal salinity excursion of 30‰ for one week (early harvest) and five weeks (late harvest); n=6, $LSD_{0.05}$=1.62, MSE=1.98.

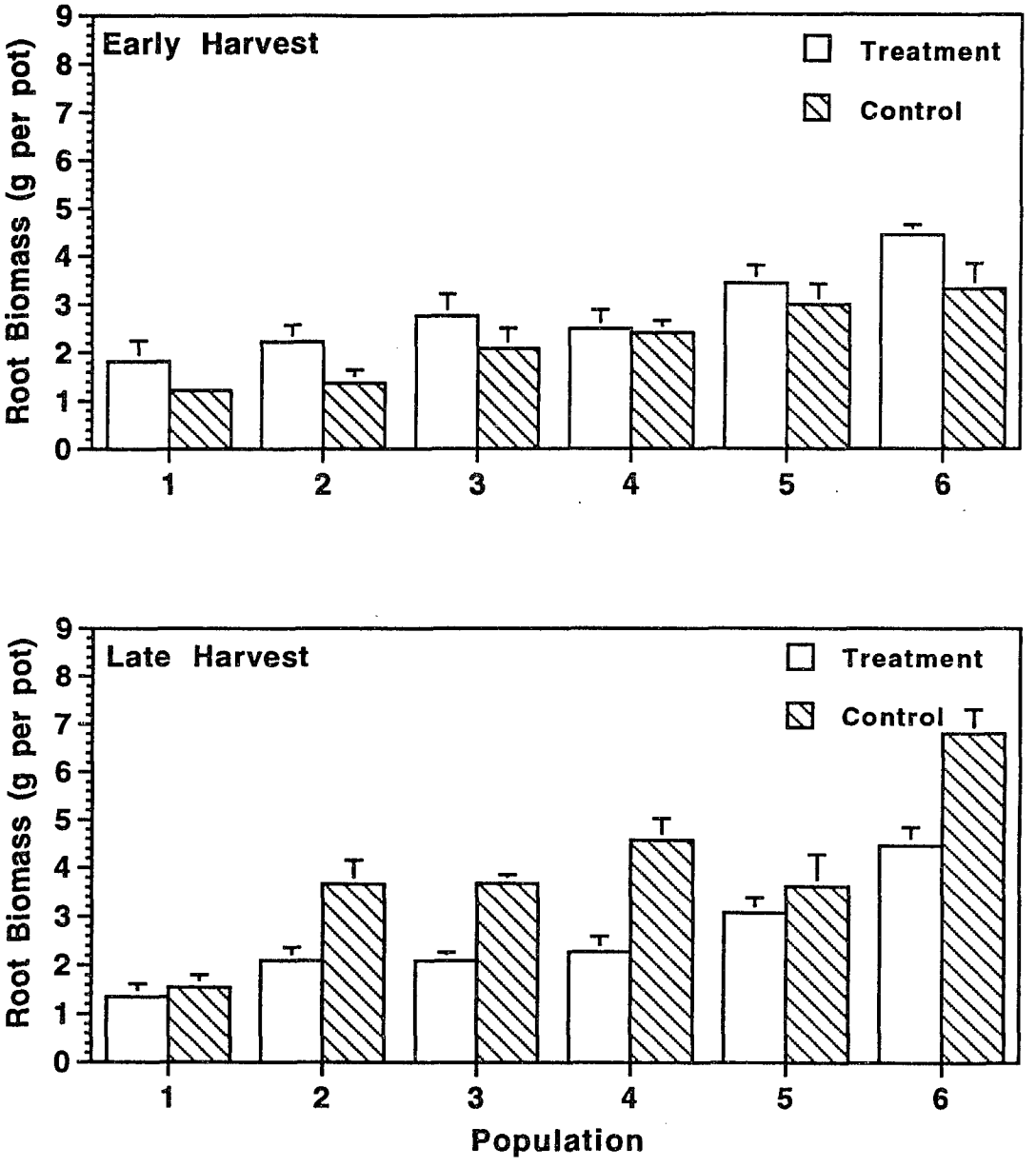

Figure 5.17. Mean (± std err) root biomass (g per pot) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Spartina alterniflora when subjected to a sublethal salinity excursion of 30‰ for one week (early harvest) and five weeks (late harvest); n=6, $LSD_{0.05}$=1.04, MSE=0.81.

and 5.17). Late harvest root biomass averaged about 1.7 g per pot for the highly salt-tolerant populations compared to 3.8 g per pot for the poorly salt-tolerant populations (Figure 5.17).

Rhizome biomass also displayed significant main effects and an interaction of harvest x treatment. However, contrasts between highly salt-tolerant and poorly salt-tolerant populations yielded no significant differences for either early or late harvests. Rhizome biomass in the late harvest ranged from 1.3 to 2.0 g per pot for treatments and 1.7 to 3.0 g per pot for controls.

Root-to-shoot ratios displayed significant harvest, treatment and population main effects and a significant harvest x population interaction. Similar to what was observed in the root data, a trend of smaller root-to-shoot ratios can be observed in populations of greater salt tolerance (Figure 5.18). Contrasts within treatments and controls revealed significant differences in root-to-shoot ratios between highly salt-tolerant, intermediate salt-tolerant, and poorly salt-tolerant populations in both harvests (Figure 5.18). Late harvest root-to-shoot ratios for highly salt-tolerant populations averaged 0.16 compared to 0.32 for the poorly salt-tolerant populations. It is also interesting to note that in the early harvest, many of the treatment root-to-shoot ratios were significantly greater than their respective controls, whereas in the late harvest only the two poorly tolerant populations (5 and 6) had treatment root-to-shoot ratios that remained significantly elevated above the controls (Figure 5.18).

DISCUSSION

Differences in biomass partitioning between highly salt-tolerant and poorly salt-tolerant populations varied depending on the species. In the late harvest (five weeks after exposure to sublethal salinity levels) highly salt-tolerant populations of Panicum hemitomon had significantly greater covariable-adjusted total plant biomass than poorly salt-tolerant populations, whereas for Spartina patens, only population 1 had greater total plant biomass than population 6, and for Spartina alterniflora, there were no significant

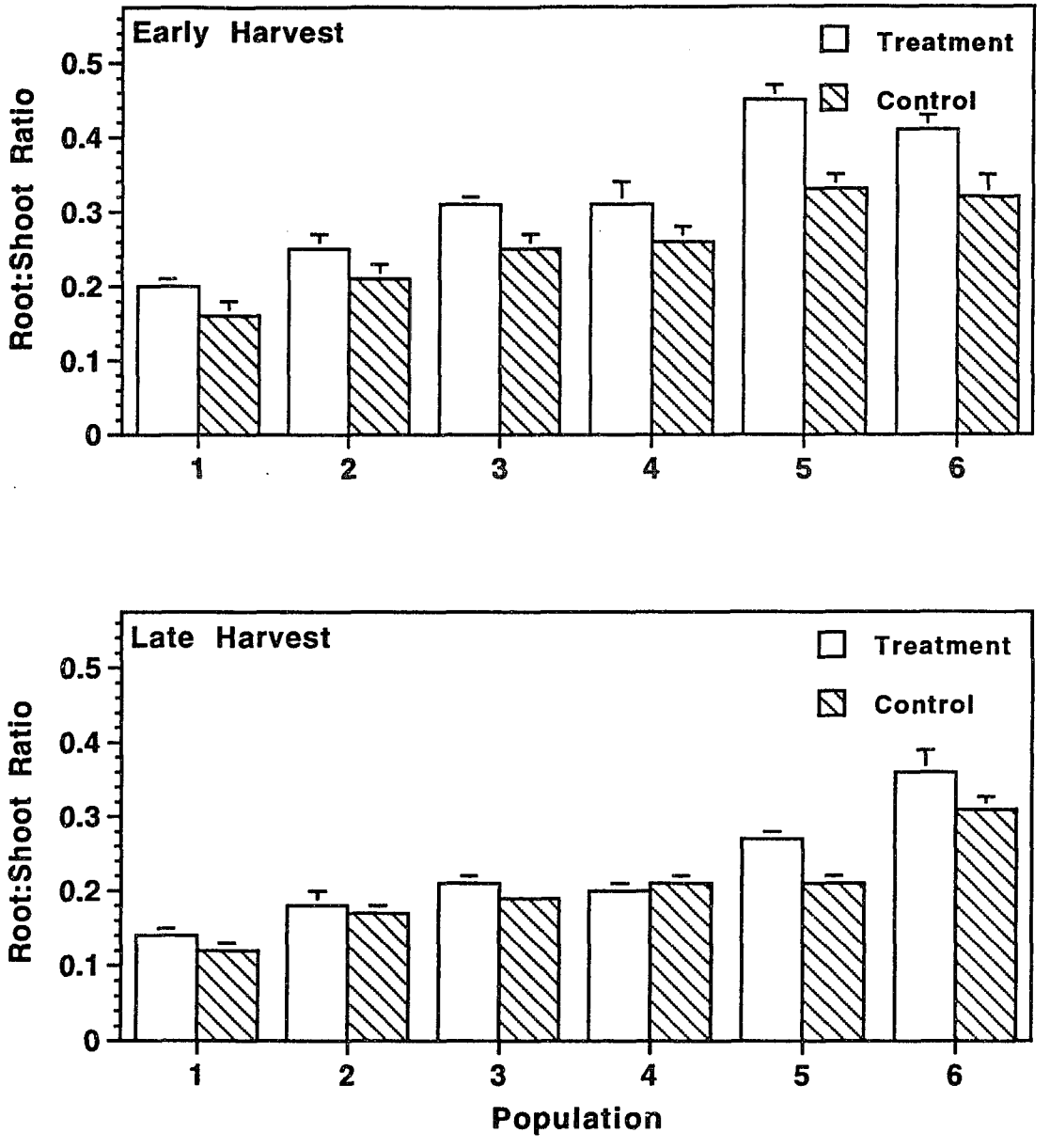

Figure 5.18. Mean (± std err) root:shoot ratio (root biomass/total aboveground biomass) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Spartina alterniflora when subjected to a sublethal salinity excursion of 30‰ for one week (early harvest) and five weeks (late harvest); n=6, $LSD_{0.05}$=0.052, MSE=0.002.

differences in covariable-adjusted total plant biomass between highly salt-tolerant and poorly salt-tolerant populations.

A similar pattern of species differences was observed in aboveground biomass. Live and total aboveground biomass were significantly greater in highly salt-tolerant populations of Panicum hemitomon in both harvests, significantly greater only in population 1 versus population 6 for late harvest Spartina patens, and not significantly different between highly salt-tolerant and poorly salt-tolerant populations of Spartina alterniflora populations in either harvest. These results indicate that any relationship between salt tolerance and plant biomass, or plant production, under salinity stress is most apparent in the fresh marsh dominant, Panicum hemitomon, less apparent in the brackish marsh dominant, Spartina patens, and totally lacking in the salt marsh dominant, Spartina alterniflora. Furthermore, the total plant biomass of the Panicum controls generally paralleled those of the respective salinity treatments, supporting the notion that intrinsic rates of growth in the absence of salinity may be a useful indicator of salt tolerance in non-halophytes, such as Panicum (Greenway and Munns 1980; Munns and Termaat 1986; Munns 1993).

The biomass results from this chapter also generally agree with the results from the previous chapter on photosynthesis and growth response, where it was observed that the sublethal salinity photosynthetic response in Panicum hemitomon could better differentiate population salt tolerance than in the two Spartina species. Pezeshki et al. (1987) reported that a sudden salinity increase in the range of 10‰ to 12‰ resulted in severe injury and tissue death of Panicum hemitomon plants within five days. McKee and Mendelssohn (1989) reported that a more gradual increase in salinity allowed Panicum to survive higher salinities of up to 9.4‰ for periods as long as 35 days. This agrees with the results from the salinity screening experiments of this research (Chapter 3), where weekly stepwise salinity increases of 2‰ allowed populations of Panicum to survive salinities in the range of 7.6‰ to 12‰ before 50% death of aboveground tissue

resulted. As stated in the previous chapter, species differences between the two Spartinas and Panicum likely reflect the fact that continued exposure of Panicum, a fresh marsh plant, to what was initially a sublethal salinity level had a cumulative negative effect through time as salts apparently continued to accumulate in its leaf tissue, whereas Spartina patens and Spartina alterniflora, brackish and salt marsh dominants, appeared better able to acclimate to some degree to these sublethal salinity levels through time. Therefore, in addition to actual salinity level, duration of exposure to elevated salinity in Panicum hemitomon appears to be an important factor affecting growth and survival.

The proportion of dead aboveground biomass was significantly increased in late harvest poorly salt-tolerant populations of Panicum hemitomon, as was also the case for Spartina alterniflora, but not Spartina patens. It is likely that the sublethal salinity level of 20‰ was not high enough to cause sufficient stress in the Spartina patens salinity treatments to significantly increase the amount of dead tissue, especially in light of the fact that controls averaged approximately 3% - 7% dead aboveground biomass by weight compared to only 5% - 8‰ dead aboveground biomass in the treatments. Similarly, Pezeshki (1991) did not detect gas exchange differences between two populations of Spartina patens of varying salt tolerance history until salinities reached 25‰.

As was the case for aboveground biomass in Panicum hemitomon, root biomass and total belowground biomass of Panicum hemitomon also showed a trend of greater biomass in the more salt-tolerant populations. Conversely, in Spartina alterniflora there was a distinctive significant trend in treatments and controls of greater belowground allocation in the more poorly salt-tolerant populations. Root-to-shoot ratios verified this tendency, with the poorly salt-tolerant populations having root-to-shoot ratios that were double those of the highly salt tolerant populations.

Observed increases in the root-to-shoot ratios of plants under salinity stress are typically attributed to shoot growth being more adversely affected by the salt stress than root growth, such that an increase in the root-to-shoot ratio results (Munns and Termaat

1986). In this study, we anticipated that the more poorly salt-tolerant populations would display increased root-to-shoot ratios due to a proportionately larger decrease in shoot growth relative to root growth under salt stress. Although this pattern of increased root-to-shoot ratios in more poorly salt-tolerant populations was observed to varying degrees in all three species, the underlying reason for the increased root-to-shoot ratio in poorly salt-tolerant Spartina alterniflora populations was not due to a more adverse effect of salinity on shoot growth; it was due to greater root growth.

In Spartina patens only early harvest poorly salt-tolerant populations had significantly larger root-to-shoot ratios, which was mainly due to less aboveground biomass as expected, not to any difference in root biomass other than that observed in population 5. In Panicum hemitomon significantly greater root-to-shoot ratios were observed only in the late harvest of the poorly salt-tolerant population 5, but not in population 6, which was not different from that of population 1. Furthermore, it should be restated that in Panicum hemitomon the poorly salt-tolerant populations had significantly less root biomass than the highly salt-tolerant populations, so any increase in root-to-shoot ratios in poorly salt-tolerant Panicum populations was due to a proportionately larger decrease in aboveground biomass.

The relationship in Spartina alterniflora root and belowground biomass allocation is interesting since the differences observed in root-to-shoot ratios were due to population differences in root growth, not population differences in aboveground growth. Controls and treatments of highly salt-tolerant populations both had relatively low root biomass and low root-to-shoot ratios. However, the poorly salt-tolerant populations had greater root biomass, with salinity resulting in root-to-shoot ratios that were significantly elevated above their respective controls. The reason for proportionately lower root biomass in the more highly salt-tolerant Spartina alterniflora populations under both non-saline and saline conditions is elusive. One can only speculate that for a more limited root system to be able support a comparable amount of

aboveground biomass, these populations may have root systems that are, in some way, more efficient at nutrient and water acquisition (Flowers et al. 1977; Blum 1988).

The importance of plant size attributes in Panicum hemitomon as being a potential factor in salt tolerance was recognized in our previous lethal salinity level screening studies on a large number of populations, and remains important based on the results of this study when exposure was maintained at a constant sublethal salinity level. The exact mechanism of this association between size and salt tolerance in Panicum hemitomon remains unknown, but it can be speculated that greater biomass results in more mature tissue available for translocation and storage of salts (Greenway and Munns 1980; Munns and Termaat 1986; Munns 1993). Total plant biomass and belowground biomass components in Panicum controls were also significantly greater in highly salt-tolerant populations compared to poorly salt-tolerant populations, indicating that in Panicum, growth of genotypes in the absence of salt (intrinsic rates of growth) may be of value in assessing salt tolerance. Rawson et al. (1988) similarly found that growth of barley and wheat genotypes of known salt tolerance in the absence of salt stress was a fairly good indicator of salt tolerance. Greenway and Munns (1980) further state that in non-halophytes, genotypes of low intrinsic rates of growth are disadvantaged at high salinities.

In summary, sublethal salinity biomass responses of Panicum showed stronger associations with known salt tolerance than what was observed in Spartina patens and Spartina alterniflora. In Panicum, greater plant biomass was associated with known salt tolerance in both salt-stressed and control plants indicating that intrinsic rates of growth in the absence of salinity may be a useful marker for future salinity screenings in this species. Highly salt-tolerant populations of Spartina alterniflora had significantly less root biomass than poorly salt-tolerant populations although aboveground biomass was similar, thereby resulting in significantly lower root-to-shoot ratios in the highly salt-tolerant populations. The proportion of dead to total aboveground biomass was

generally associated with known salt tolerance in Panicum hemitomon and Spartina alterniflora by the late harvest, but not in Spartina patens, probably because 20‰ was not stressful enough to elucidate strong population differences in salinity tolerance.

Chapter 6

Investigations of Factors Associated with Intraspecific Variation in Salt Tolerance in Panicum hemitomon, Spartina patens and Spartina alterniflora. III. Leaf Water Potential, Osmotica, and Cation Concentrations

INTRODUCTION

Our previous research has shown that significant intraspecific variation in salt tolerance (lethal salinity level) exists in Gulf Coast populations of Panicum hemitomon, Spartina patens, and Spartina alterniflora, dominant emergent macrophytes of fresh, brackish, and salt marsh plant communities, respectively. This manuscript is the third of a three-part series in which plant photosynthetic and growth responses, biomass partitioning, and plant water relations and osmotic adjustment associated with differential salt tolerance were investigated within each of these three coastal marsh dominants when populations of known varying degrees of salt tolerance were exposed to a sublethal salinity excursion.

In the first two parts of this series results of plant photosynthesis, growth responses and biomass partitioning were presented. In all three species, plant photosynthetic rates in populations previously classified as highly salt-tolerant were greater than those of populations classified as poorly salt tolerant one week after exposure to sublethal salinity levels, and in Panicum this trend continued into the late harvest (five weeks). Water use efficiencies in late harvest Panicum populations were also greater in the more salt-tolerant populations compared to the two Spartina species, which did not show significant population differences.

Relationships between plant biomass and salt tolerance were most apparent in the fresh marsh dominant, Panicum hemitomon, less apparent in the brackish marsh dominant, Spartina patens, and generally lacking in Spartina alterniflora, the salt marsh

dominant. In Panicum, plant biomass responses generally followed the trends displayed by plant photosynthetic response, with highly salt-tolerant populations producing more aboveground, belowground and total biomass than poorly salt-tolerant populations. Root-to-shoot ratios were significantly elevated to varying degrees in poorly salt-tolerant populations of all three species, but more so in Panicum. Interestingly, in Spartina alterniflora increased root-to-shoot ratios were observed in treatments as well as controls of poorly salt-tolerant populations and were due largely to increased root biomass, not decreased shoot biomass. The proportion of dead aboveground biomass was significantly greater in poorly salt-tolerant populations than highly salt-tolerant populations of Panicum and Spartina alterniflora, but not Spartina patens, possibly because the sublethal salinity selected for this species appeared to have been relatively less stressful than those selected for Panicum and Spartina alterniflora.

This manuscript is the third part of this series and reports the results of plant water relations, osmotica and cation concentrations within each of these species at sublethal salinity levels. Investigations of plant water relations and tissue contents are useful in understanding salinity tolerance by revealing potential population differences in plant water status, ion uptake or exclusion mechanisms, and synthesis and accumulation of osmotica or stress metabolites that may be associated with superior salt tolerance.

MATERIALS AND METHODS

Plant material

Population selection was based on our previous salinity screening research in which we identified intraspecific variation in lethal salinity level in each of these three species as described in Part I (Chapter 4) of this series. Based on these results, we selected two of the most salt-tolerant genotypes, two genotypes of intermediate salt tolerance, and two of the least salt-tolerant genotypes within each species. In this series we refer to the highly salt-tolerant populations within each species as populations 1 and 2, the two populations of intermediate salinity tolerance as populations 3 and 4, and the

two poorly salt-tolerant populations as populations 5 and 6. The corresponding alphabetic population code for these selected populations, as used in the previous research on screening for intraspecific variation in salt tolerance is described in Part I. The respective mean lethal salinity levels for populations 1 through 6 of each species are as follows: Panicum hemitomon populations 1 (12‰), 2 (11.2‰), 3 (10‰), 4 (9.6‰), 5 (7.6‰), 6 (7.6‰); Spartina patens populations 1 (93‰), 2 (89‰), 3 (83‰), 4 (81‰), 5 (66‰), 6 (63‰); Spartina alterniflora populations 1 (115‰), 2 (115‰), 3 (107‰), 4 (101‰), 5 (93‰), 6 (93‰).

Plant material was obtained by vegetatively propagating the selected genotypes from the stock populations, which had been maintained under non-saline conditions in a temperature controlled glasshouse for six to eight vegetative generations prior to the beginning of this experiment as described in Part I.

Experimental design

The experimental design for each species was a factorial randomized block design of six replicates, or blocks, that blocked on bench variation within the growth chamber. The three species experiments were run sequentially, not simultaneously. For each species the following 6 x 2 x 2 factorial arrangement of main effects was tested: population effect (six populations ranging from high to low salinity tolerance), treatment effect (sublethal salinity level versus control at non-saline conditions), harvest effect (early harvest one week after exposure to sublethal salinity level and late harvest five weeks after exposure to sublethal salinity level), yielding a total of 144 experimental units for each species experiment.

All experiments were conducted in a temperature-controlled EGC walk-in growth chamber set to 16 hr daylength at 30 C and an 8 hr dark period at 24 C as described in Part I. Depending on the size of the plants, two to four young stems were planted per pot and placed inside larger reservoir pots that contained the treatment bathing solution at the desired salinity.

Each experiment consisted of two harvests: an early harvest one week after exposure to the designated sublethal salinity level and a late harvest after four additional weeks (five weeks total exposure to the sublethal salinity level). Additional information on variables measured in each harvest is provided in Part I.

Salinity regime

The sublethal salinity level varied depending on the species as follows: Panicum hemitomon, 4‰; Spartina patens, 20‰; and Spartina alterniflora, 30‰. All experimental units were initially maintained in a treatment bathing solution of half-strength Hoagland's nutrient solution (Hoagland and Arnon 1950) at 0‰ salinity for a two week period following transplanting. Salinity was then increased stepwise over a relatively short time interval until the targeted sublethal salinity level was reached using a commercial sea salts mix (Instant Ocean®; Aquarium Systems, Mentor, Ohio) in half-strength Hoagland's as described in Part I. Controls were kept at 0‰ salinity in half-strength Hoagland's except for the Spartina alterniflora controls, which were provided with 1‰ sea salt in half-strength Hoagland's. All bathing solutions were drained and replaced at weekly intervals and salinities were checked twice weekly throughout each experiment.

Analytical techniques

Leaf xylem pressure was measured on the second fully expanded leaf of a randomly selected mature stem. Leaves were cleanly severed from the plant, inserted into a pressure equilibration chamber (Soil Moisture Equipment Co., Model # 3005) and the pressure gradually increased until xylem fluid was observed to exude according to the methods of Scholander et al. (1965).

At harvest, green leaf tissue was clipped from the stem, lightly rinsed in deionized water, placed in sterile plastic bags that were placed inside larger plastic bags that contained sufficient water to cover the specimen bag, and then were immediately frozen in liquid nitrogen. Tissue was maintained frozen in liquid nitrogen until all tissue

samples were collected for a given harvest (two days) and then freeze-dried (lyophilized) using a Labconco freeze drier. Lyophilized leaf tissue was maintained in a dessicator with desiccant under a vacuum until it was clipped into small (1 - 2 mm) pieces with stainless steel scissors for analysis.

Leaf cation concentrations were determined with an inductively coupled argon plasma emission spectrometer (Jarrell-Ash Atom Comp series 800) after digestion of 0.25 g lyophilized tissue in 3.0 ml concentrated nitric acid at 130° C for six hours, which was brought up to 35.0 ml final volume with distilled-deionized water.

Leaf carbohydrates were extracted from lyophilized tissue after homogenizing in 95% HPLC-grade ethanol for 45 seconds followed by a 30 minute extraction period (adapted from Picha 1985). Samples were then vortexed for 30 seconds and filtered through a Whatman #4 qualitative filter, which was rinsed several times with HPLC-grade ethanol as the sample extract was brought up to volume (Picha 1985). Sample extracts were then filtered through a 0.2μ nylon syringe filter prior to injection. Sucrose, fructose, glucose, and maltose were determined by high-performance liquid chromatography (HPLC) with a Bio-Sil Amino 5S column using an 80% CH_3CN: 20% H_2O mobile phase and refractive index detection (Picha 1985). Recoveries of sugar standards using this technique ranged from 85% to 88%.

Leaf proline and glycinebetaine were extracted using a modification of Gorham (1984), whereby lyophilized tissue was homogenized in 70% HPLC-grade methanol for 45 seconds followed by a 30 minute extraction period. Sample extracts were then vortexed and filtered through a 1.2 μ cellulose syringe filter, which was rinsed with additional HPLC-grade methanol, and brought up to volume. A pipetted aliquot of the methanol extract was then dried under vacuum at 40 C with a Labconco Rapidvap (model 79000) and rehydrated with a ultra-pure HPLC-grade water. The water extract (2.0 ml) was then allowed to exchange with a 1:2 ratio (0.5 ml:1.0 ml) of cation to anion exchange resin for 30 minutes and filtered through a 0.2μ nylon syringe filter. Cation

exchange resin was Amberlite IRC 50 regenerated as H^+ using 0.5 N HCL. Anion exchange resin was Amberlite IRA 401 regenerated as CH_3COO^- using 0.5 N sodium acetate. It should be noted that regeneration of the anion exchange resin as OH^- (0.5 N NaOH) resulted in nearly complete loss (>90%) of proline, whereas regeneration with acetate resulted in recoveries of 96% to 98% for proline and glycinebetaine. Leaf proline and glycinebetaine were determined by high-performance liquid chromatography (HPLC) with a Whatman SCX (strong cation exchange) silica based (10μ particle) column using a buffer of 50 mol m^{-3} KH_2PO_4 (pH 5.0) for the mobile phase at a flow rate of 1.0 ml min^{-1} and a U.V. detector at 200 nm (modified from Gorham 1984). Panicum hemitomon samples contained an unidentified compound that interfered with the accurate determination of glycinebetaine. Therefore, only proline concentrations are reported for Panicum hemitomon.

Data analysis

Data was analyzed as a factorial randomized block design. Analysis of variance (ANOVA) was used to test for significant main effects and interactions using SAS (SAS 1989; Steel and Torrie 1980). A significance (alpha) level of 0.05 was used for all analyses unless otherwise stated. Single degree of freedom contrasts were used to make a priori comparisons between populations of different degrees of salt tolerance. All data were tested for meeting the assumptions of normality and homogeneity of variance by using a combination of the Shapiro-Wilk test statistic for tests of normality and the Bartlett test for tests of homogeneity of variance (SAS 1989). Data that did not meet these assumptions were transformed until assumptions were met.

RESULTS

Panicum hemitomon

Leaf xylem pressure potential displayed a significant treatment main effect and a significant harvest x treatment interaction. Early harvest mean leaf xylem pressures were -1.6 and -2.4 MPa for controls and salinity treatments, respectively, compared to -2.0

and -2.2 MPa for late harvest controls and treatments (Figure 6.1). Contrasts within early harvest treatments revealed that the poorly salt-tolerant populations (populations 5 and 6) had significantly lower leaf xylem pressures (-2.7 to -3.0 MPa) than the highly salt-tolerant populations (populations 1 and 2; -2.1 to -2.2 MPa), whereas contrasts within controls showed no significant differences in either harvest.

In the late harvest, population 5 (poorly salt tolerant) displayed nearly complete chlorosis/necrosis of plant tissue in four of its six treatment experimental units. The remaining two experimental units had adequate green tissue for leaf carbohydrate analyses, but not cation or proline analyses. One experimental unit of population 6 (poorly salt-tolerant), and one experimental unit of population 2 (highly salt tolerant) also lacked sufficient green leaf tissue for any analytical determinations. Quantification of live and dead aboveground biomass is provided in the previous chapter (Chapter 5; Part II of this series).

Leaf Na displayed significant harvest and treatment main effects and a significant harvest x treatment interaction, but not a significant population effect. Late harvest Na concentrations were greater then early harvest concentrations, and this increase was greater in the salinity treatments than in the controls (Figure 6.2). Early harvest leaf Na concentrations for controls and salinity treatments, respectively, were 0.090 mmol g^{-1} d wt (mmol per gram dry weight of plant tissue) and 0.358 mmol g^{-1} d wt compared to 0.272 mmol g^{-1} d wt and 0.764 mmol g^{-1} d wt, respectively, for the late harvest.

Significant treatment and population main effects were detected in leaf potassium, and in all interactions, with the exception of harvest x population (Figure 6.3). Contrasts between highly salt-tolerant and poorly salt-tolerant populations showed no significant differences within controls of either harvest, nor within late harvest treatments. However, within early harvest treatments the poorly salt-tolerant populations had significantly greater leaf K than the highly salt-tolerant populations, mainly due to the relatively high leaf K value of population 5 (Figure 6.3).

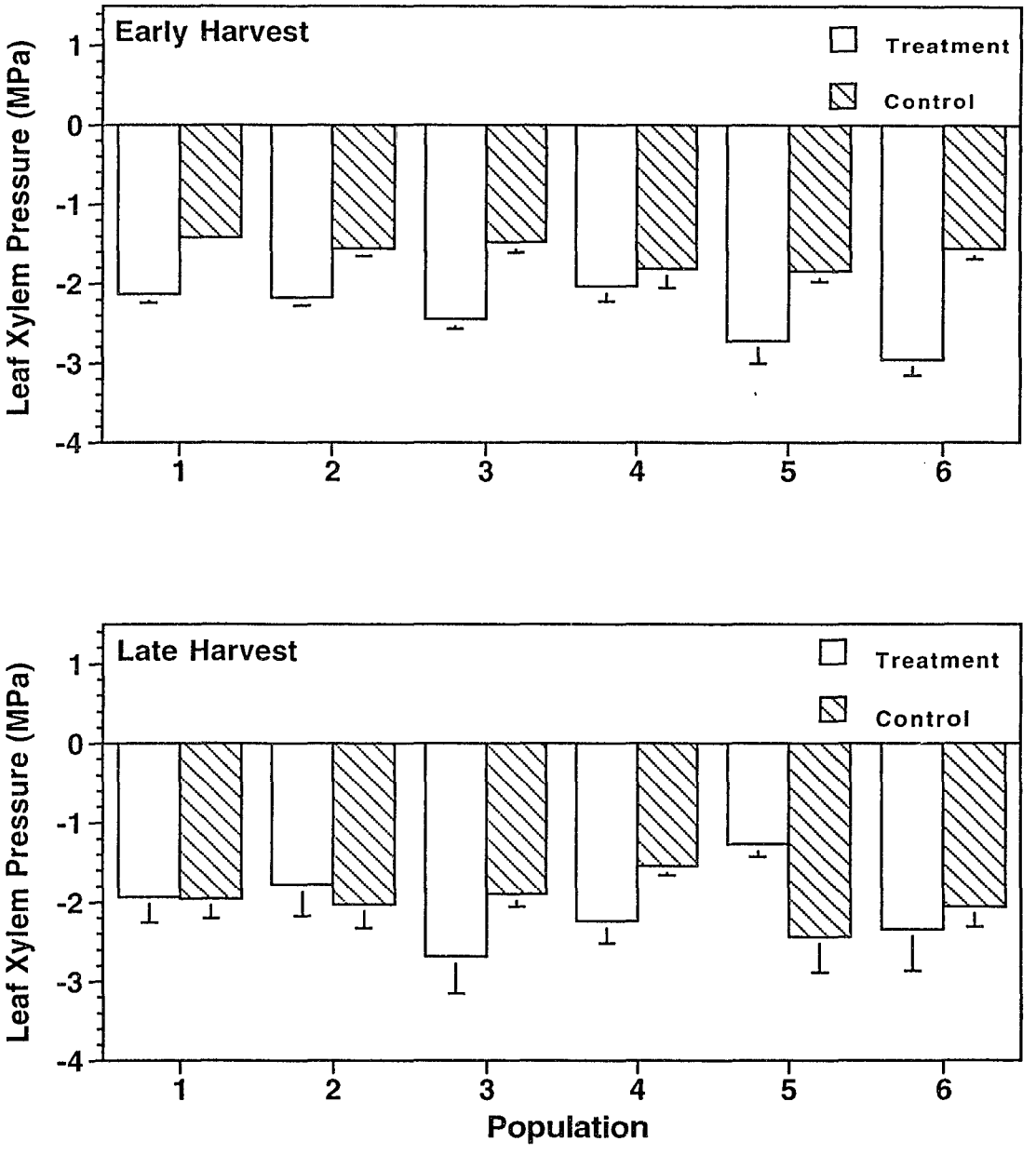

Figure 6.1. Mean (± std err) midday leaf xylem pressure (MPa) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Panicum hemitomon when subjected to a sublethal salinity excursion of 4‰ for one week (early harvest) and five weeks (late harvest); n=6 except for the following late harvest salinity treatments: n=5 for population 2, n=2 for population 5 and n=5 for population 6; $LSD_{0.05}$=7.71; MSE=40.76.

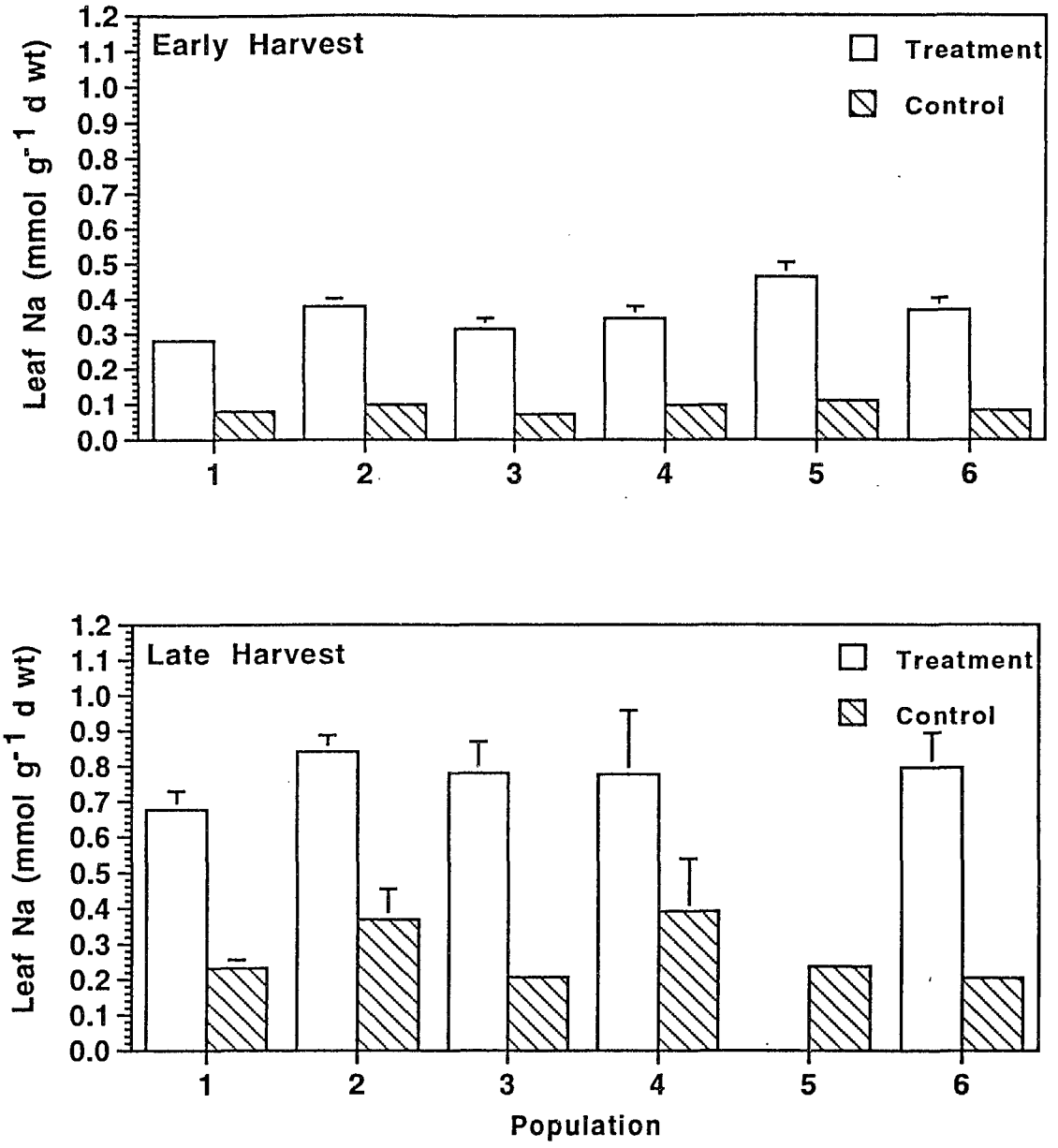

Figure 6.2. Mean (± std err) leaf Na concentration (mmol g^{-1} d wt) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Panicum hemitomon when subjected to a sublethal salinity excursion of 4‰ for one week (early harvest) and five weeks (late harvest); n=6 except for the following late harvest salinity treatments: n=5 for population 2, n=0 for population 5 and n=5 for population 6; $LSD_{0.05}$=0.181; MSE=0.021.

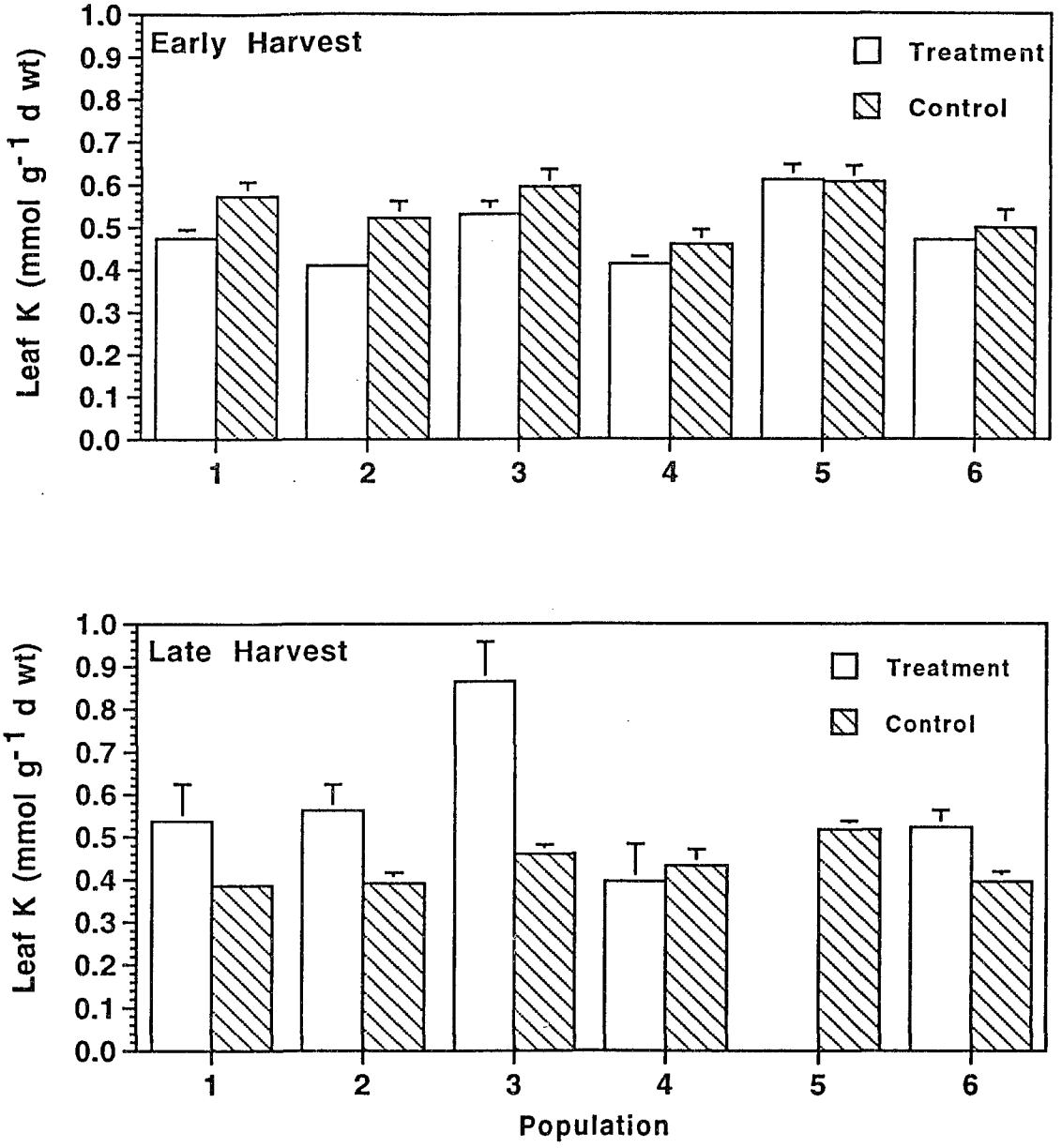

Figure 6.3. Mean (± std err) leaf K concentration (mmol g^{-1} d wt) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Panicum hemitomon when subjected to a sublethal salinity excursion of 4‰ for one week (early harvest) and five weeks (late harvest); n=6 except for the following late harvest salinity treatments: n=5 for population 2, n=0 for population 5 and n=5 for population 6; $LSD_{0.05}$=0.125; MSE=0.010.

Leaf Na:K ratio displayed significant main effects and all two-way interactions (Figure 6.4). Contrasts within early and late harvests showed no significant differences between highly salt-tolerant and poorly salt-tolerant treatments or controls. Late harvest controls had leaf Na:K ratios that ranged from 0.45 to 0.96 mmol g^{-1} d wt compared to a range of 1.32 to 1.98 mmol g^{-1} d wt in the treatments (Figure 6.4).

Leaf calcium and leaf magnesium both displayed significant treatment and population main effects only (Tables 6.1 and 6.2). The average leaf Ca of population 1 (0.067 mmol g^{-1} d wt) was significantly greater than that of population 6 (0.058 mmol g^{-1} d wt). Salinity treatment leaf Ca concentrations averaged 0.065 mmol g^{-1} d wt compared to 0.058 mmol g^{-1} d wt for controls (Table 6.1). Contrasts between highly salt-tolerant populations and poorly salt-tolerant populations failed to detect any significant differences in leaf Mg, with the highly salt-tolerant populations having intermediate values (Table 6.2). Leaf Mg concentrations averaged 0.131 mmol g^{-1} d wt in the salinity treatments compared to 0.094 mmol g^{-1} d wt in controls.

Leaf total cation concentration (sum of Na, K, Ca, and Mg) showed significant harvest, treatment and population main effects and a significant harvest x treatment interaction (Figure 6.5). Total cation concentrations in controls averaged 0.784 mmol g^{-1} d wt and 0.853 mmol g^{-1} d wt for the early and late harvests, respectively, whereas treatments displayed an increase in total cation concentration from 1.040 mmol g^{-1} d wt in the early harvest to 1.546 mmol g^{-1} d wt in the late harvest. Contrasts within treatments showed that the poorly salt-tolerant populations in the early harvest had significantly greater total cation concentrations than the highly salt-tolerant populations, whereas there were no significant differences within controls of either harvest (Figure 6.5). The total cation concentration of the population 5 salinity treatment was particularly high in the early harvest, resulting in insufficient green tissue for analysis in all experimental units of this population by the late harvest.

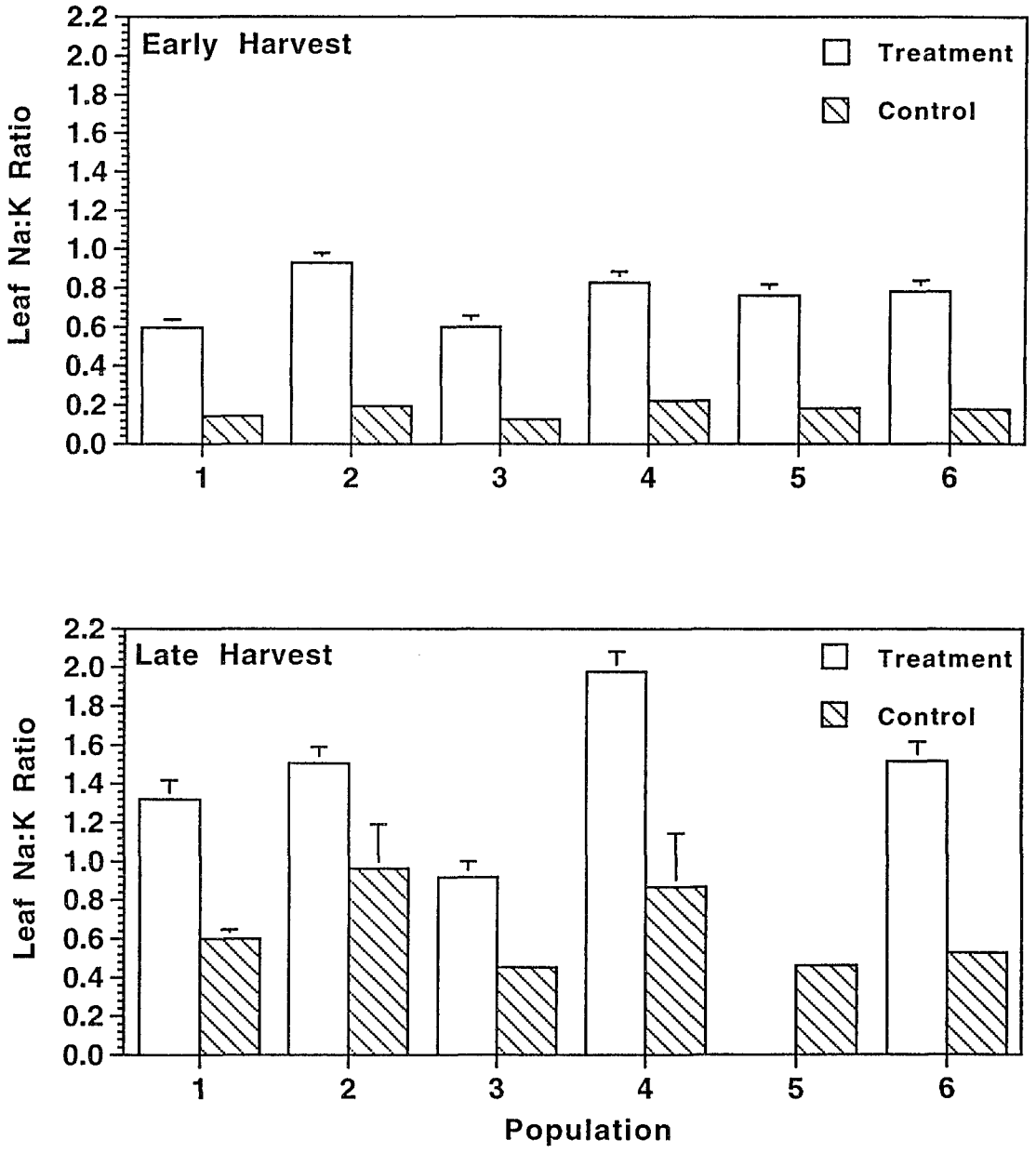

Figure 6.4. Mean (± std err) leaf Na:K ratio in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Panicum hemitomon when subjected to a sublethal salinity excursion of 4‰ for one week (early harvest) and five weeks (late harvest); n=6 except for the following late harvest salinity treatments: n=5 for population 2, n=0 for population 5 and n=5 for population 6; $LSD_{0.05}$=0.256; MSE=0.042.

Table 6.1. Mean (± standard error in parentheses) leaf Ca concentration (mmol g^{-1} d wt) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Panicum hemitomon, Spartina patens, and Spartina alterniflora when subjected to a sublethal salinity excursion of one week (early harvest) and five weeks (late harvest); n=6 except for the following Panicum hemitomon late harvest salinity treatments: n=5 for population 2, n=0 for population 5 and n=5 for population 6; Panicum hemitomon $LSD_{0.05}$=0.0147, MSE=0.00014; Spartina patens $LSD_{0.05}$=0.0089, MSE=0.0001; Spartina alterniflora $LSD_{0.05}$=0.0215, MSE=0.00034.

		Panicum hemitomon		Spartina patens		Spartina alterniflora	
		Treatment	Control	Treatment	Control	Treatment	Control
Early Harvest							
Population	1	0.071 (0.005)	0.056 (0.003)	0.055 (0.003)	0.047 (0.001)	0.093 (0.005)	0.085 (0.004)
	2	0.067 (0.005)	0.064 (0.002)	0.067 (0.005)	0.073 (0.004)	0.084 (0.004)	0.063 (0.016)
	3	0.065 (0.003)	0.047 (0.003)	0.055 (0.002)	0.052 (0.004)	0.093 (0.007)	0.092 (0.007)
	4	0.067 (0.002)	0.060 (0.004)	0.055 (0.001)	0.046 (0.002)	0.075 (0.004)	0.080 (0.004)
	5	0.065 (0.003)	0.061 (0.002)	0.080 (0.007)	0.061 (0.003)	0.064 (0.005)	0.079 (0.004)
	6	0.061 (0.003)	0.049 (0.001)	0.049 (0.003)	0.043 (0.003)	0.078 (0.003)	0.073 (0.009)
Late Harvest							
Population	1	0.081 (0.007)	0.060 (0.003)	0.059 (0.002)	0.056 (0.005)	0.189 (0.009)	0.118 (0.011)
	2	0.075 (0.002)	0.063 (0.006)	0.063 (0.003)	0.065 (0.004)	0.165 (0.007)	0.097 (0.005)
	3	0.055 (0.005)	0.048 (0.004)	0.054 (0.003)	0.041 0.004)	0.183 (0.006)	0.145 (0.008)
	4	0.056 (0.013)	0.059 (0.006)	0.069 (0.003)	0.050 (0.003)	0.126 (0.012)	0.089 (0.003)
	5	— —	0.066 (0.007)	0.086 (0.005)	0.058 (0.003)	0.181 (0.011)	0.115 (0.008)
	6	0.063 (0.011)	0.060 (0.007)	0.063 (0.003)	0.048 (0.004)	0.168 (0.003)	0.122 (0.010)

Table 6.2. Mean (± standard error in parentheses) leaf Mg concentration (mmol g^{-1} d wt) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Panicum hemitomon, Spartina patens, and Spartina alterniflora when subjected to a sublethal salinity excursion of one week (early harvest) and five weeks (late harvest); n=6 except for the following Panicum hemitomon late harvest salinity treatments: n=5 for population 2, n=0 for population 5 and n=5 for population 6; Panicum hemitomon $LSD_{0.05}$=0.0253, MSE=0.00042; Spartina patens $LSD_{0.05}$=0.0057, MSE=0.00003; Spartina alterniflora $LSD_{0.05}$=0.0252, MSE=0.0005.

		Panicum hemitomon		Spartina patens		Spartina alterniflora	
		Treatment	Control	Treatment	Control	Treatment	Control
Early Harvest							
Population	1	0.117 (0.006)	0.087 (0.003)	0.044 (0.002)	0.033 (0.001)	0.145 (0.007)	0.087 (0.003)
	2	0.133 (0.009)	0.100 (0.004)	0.060 (0.002)	0.042 (0.001)	0.127 (0.007)	0.084 (0.019)
	3	0.158 (0.008)	0.100 (0.001)	0.053 (0.003)	0.041 (0.001)	0.158 (0.012)	0.124 (0.010)
	4	0.099 (0.004)	0.073 (0.002)	0.054 (0.002)	0.037 (0.002)	0.126 (0.005)	0.102 (0.003)
	5	0.160 (0.004)	0.122 (0.004)	0.063 (0.004)	0.042 (0.002)	0.108 (0.009)	0.112 (0.007)
	6	0.122 (0.004)	0.087 (0.003)	0.040 (0.001)	0.030 (0.002)	0.114 (0.005)	0.084 (0.009)
Late Harvest							
Population	1	0.139 (0.007)	0.081 (0.003)	0.054 (0.002)	0.035 (0.002)	0.218 (0.014)	0.113 (0.011)
	2	0.152 (0.003)	0.102 (0.011)	0.070 (0.003)	0.043 (0.002)	0.161 (0.007)	0.113 (0.008)
	3	0.141 (0.009)	0.095 (0.006)	0.058 (0.003)	0.036 (0.002)	0.250 (0.008)	0.143 (0.007)
	4	0.108 (0.023)	0.081 (0.017)	0.076 (0.002)	0.035 (0.002)	0.147 (0.015)	0.089 (0.002)
	5	— —	0.109 (0.009)	0.076 (0.002)	0.043 (0.003)	0.202 (0.009)	0.119 (0.005)
	6	0.125 (0.009)	0.089 (0.008)	0.079 (0.003)	0.034 (0.002)	0.207 (0.004)	0.115 (0.009)

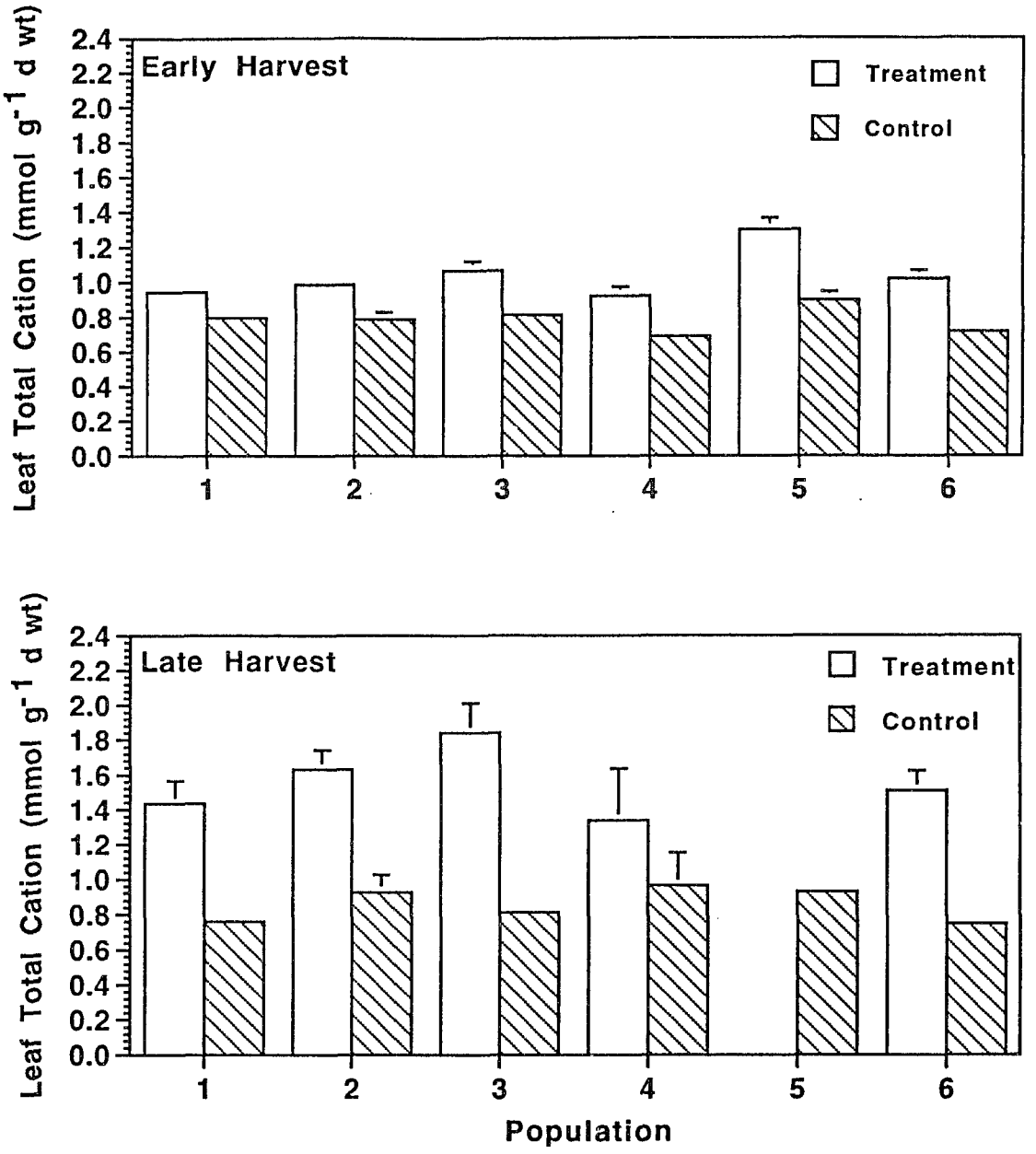

Figure 6.5. Mean (± std err) leaf total cation concentration (mmol g^{-1} d wt sum of Na, K, Ca, Mg) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Panicum hemitomon when subjected to a sublethal salinity excursion of 4‰ for one week (early harvest) and five weeks (late harvest); n=6 except for the following late harvest salinity treatments: n=5 for population 2, n=0 for population 5 and n=5 for population 6; $LSD_{0.05}$=0.291; MSE=0.055.

Leaf proline showed significant treatment and population main effects and a significant treatment x population interaction. Early harvest contrasts within treatments showed significantly greater proline in the poorly salt-tolerant populations (283 - 295 μmol g^{-1} d wt) compared to the highly salt-tolerant populations (96 - 106 μmol g^{-1} d wt), whereas there were no significant differences within controls (Figure 6.6). In the late harvest treatments, leaf proline levels of population 6 still remained significantly elevated above those of populations 1 and 2 (Figure 6.6).

Leaf sucrose displayed a significant treatment main effect only, with the salinity treatment resulting in higher sucrose concentrations. There were no significant differences between highly salt-tolerant and poorly salt-tolerant populations. Mean treatment sucrose concentrations were 50.3 μmol g^{-1} d wt compared to 29.4 μmol g^{-1} d wt for controls (Figure 6.7). There were no significant main effects or interactions in Panicum leaf fructose, glucose, or maltose. Respective mean control and treatment values were 9.04 and 6.31 μmol g^{-1} d wt for fructose, 1.01 and 22.14 μmol g^{-1} d wt for glucose, and 0.03 and 3.56 μmol g^{-1} d wt for maltose.

Spartina patens

Leaf xylem pressure displayed significant harvest, treatment and population main effects. Leaf xylem pressures averaged -2.0 and -3.0 MPa for the early harvest controls and treatments, respectively, compared to corresponding late harvest values of -1.4 and -2.6 MPa (Figure 6.8). Within treatments there was a tendency for the highly salt-tolerant populations to have significantly greater leaf xylem pressures than the poorly salt-tolerant populations in the early harvest ($P \leq 0.10$) and in the late harvest ($P \leq 0.06$), whereas no significant differences were detected within controls of either harvest (Figure 6.8).

Leaf Na concentrations showed significant harvest, treatment and population main effects and all two-way interactions. Population 2 behaved differently than population 1, although they were both classified as highly salt tolerant. The treatment

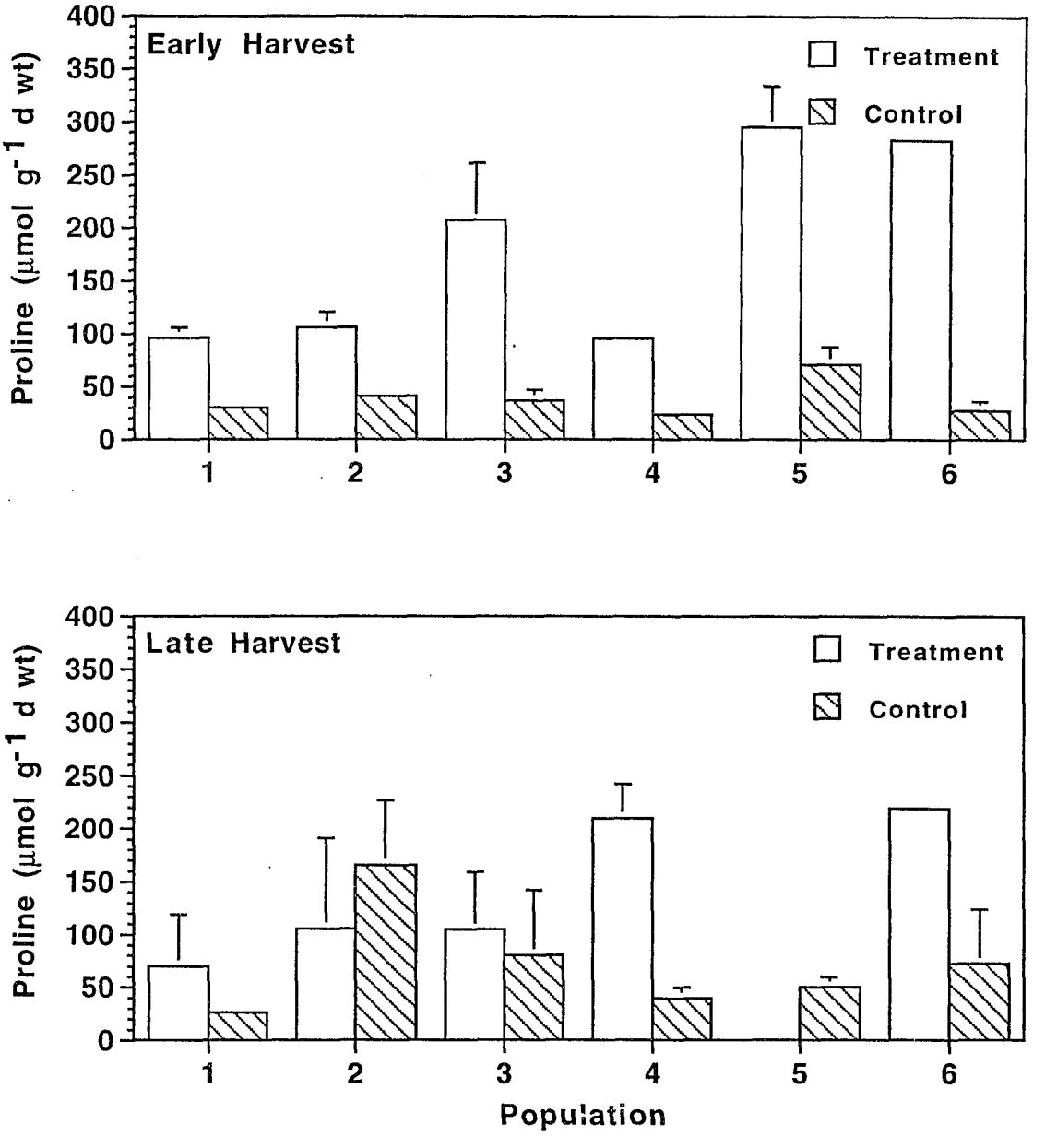

Figure 6.6. Mean (± std err) leaf proline concentration (µmol g^{-1} d wt) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Panicum hemitomon when subjected to a sublethal salinity excursion of 4‰ for one week (early harvest) and five weeks (late harvest); n=3 except n=0 for population 5 late harvest salinity treatment; $LSD_{0.05}$=107.1; MSE=3937.3.

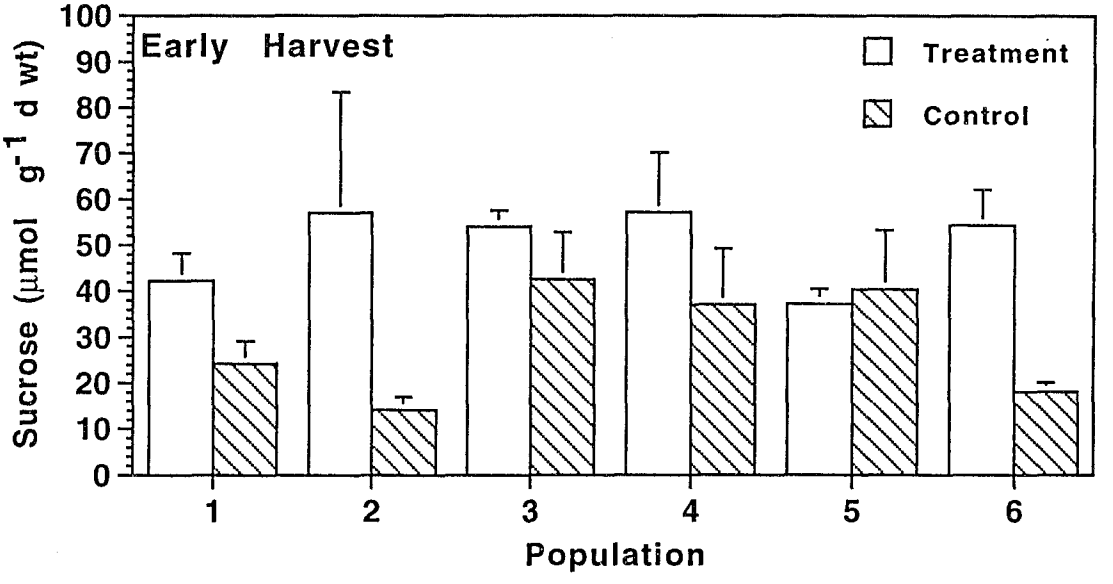

Figure 6.7. Mean (± std err) leaf sucrose concentration (µmol g^{-1} d wt) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Panicum hemitomon when subjected to a sublethal salinity excursion of 4‰ for one week (early harvest); n=3, $LSD_{0.05}$=31.25, MSE=340.6.

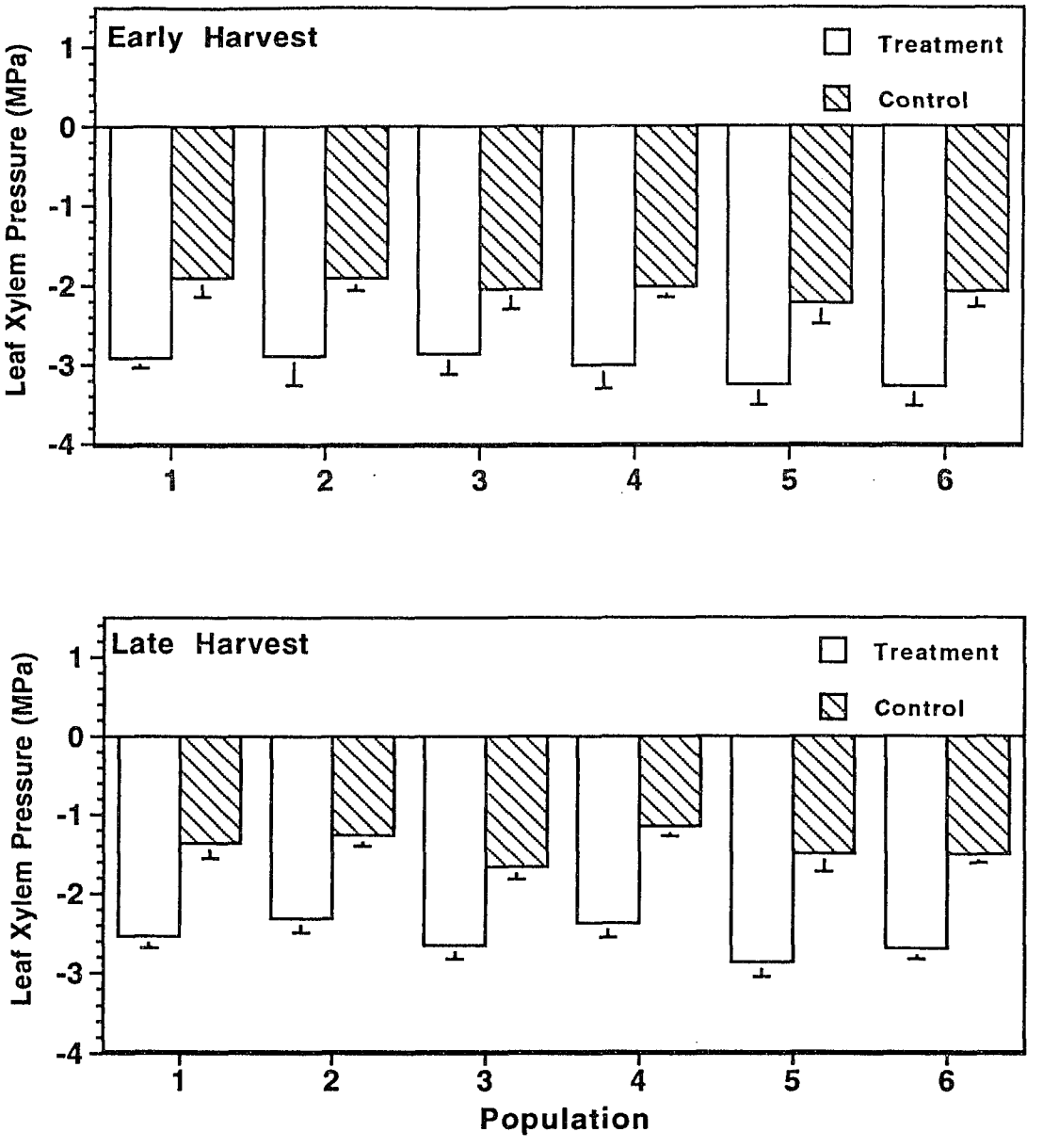

Figure 6.8. Mean (± std err) midday leaf xylem pressure (MPa) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Spartina patens when subjected to a sublethal salinity excursion of 20‰ for one week (early harvest) and five weeks (late harvest); n=6, $LSD_{0.05}$=5.08, MSE=19.73.

and control leaf Na of population 2 tended to be greater than other treatments and controls, significantly so in the early harvest. However, within treatment contrasts of population 1 versus population 6 showed that population 1 leaf Na levels were significantly lower than those of population 6 in both harvests. Furthermore, the late harvest control of population 1 also had significantly less leaf Na than the control of population 6 (Figure 6.9).

Leaf K displayed significant harvest, treatment and population main effects and a significant harvest x population interaction. Interestingly, the mean early harvest K concentrations were greater (0.680 mmol g^{-1} d wt) than mean late harvest concentrations (0.485 mmol g^{-1} d wt; Figure 6.10). Contrasts showed no significant differences between highly salt-tolerant and poorly salt-tolerant populations in the early harvest, but in the late harvest significantly greater K in poorly salt-tolerant populations (Figure 6.10).

Leaf Na:K ratios displayed significant main effects and all interactions except harvest x treatment, with the salinity treatments having larger ratios than the controls in both harvests (Figure 6.11). Controls had an average Na:K ratio of 0.49 compared to 1.29 for the treatments. Late harvest responses across populations were quite variable in controls as well as in treatments (Figure 6.11). Again, the high Na levels of population 2 were evident in this ratio. Otherwise, no pattern could be detected to differentiate highly salt-tolerant and poorly salt-tolerant populations.

Leaf Ca displayed significant treatment and population main effects and all two-way interactions. Differences between treatments and controls were not large, and in fact (although not significant) there was a tendency for population 2 controls to have greater values than population 2 treatments (Table 6.1). Contrasts within treatments and controls showed that population 1 leaf Ca was not significantly different than that of population 6 in either harvest. However, due to the influence of population 2, contrasts between highly salt-tolerant and poorly salt-tolerant populations showed that highly

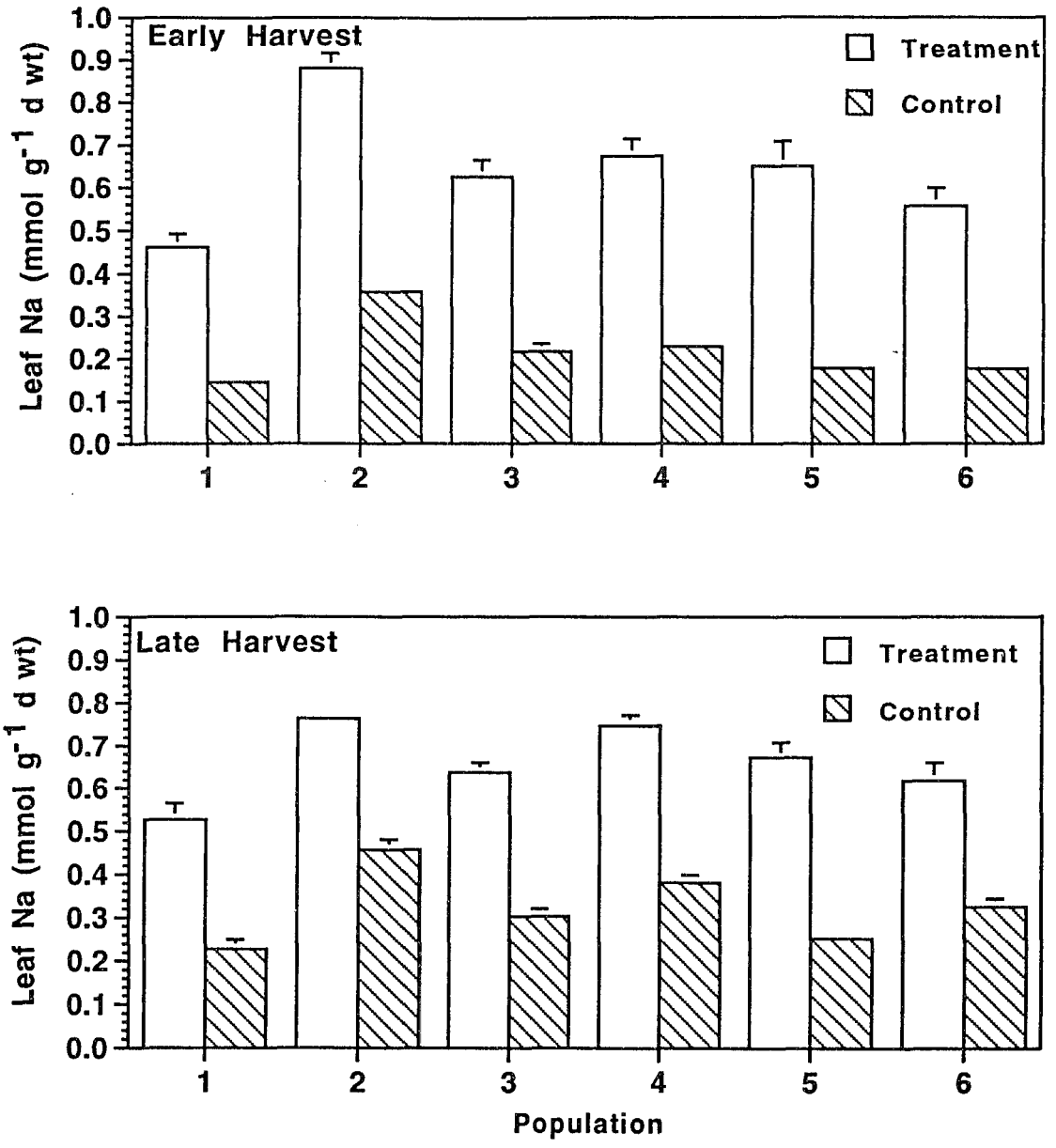

Figure 6.9. Mean (± std err) leaf Na concentration (mmol g^{-1} d wt) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Spartina patens when subjected to a sublethal salinity excursion of 20‰ for one week (early harvest) and five weeks (late harvest); n=6, $LSD_{0.05}$=0.064, MSE=0.0031.

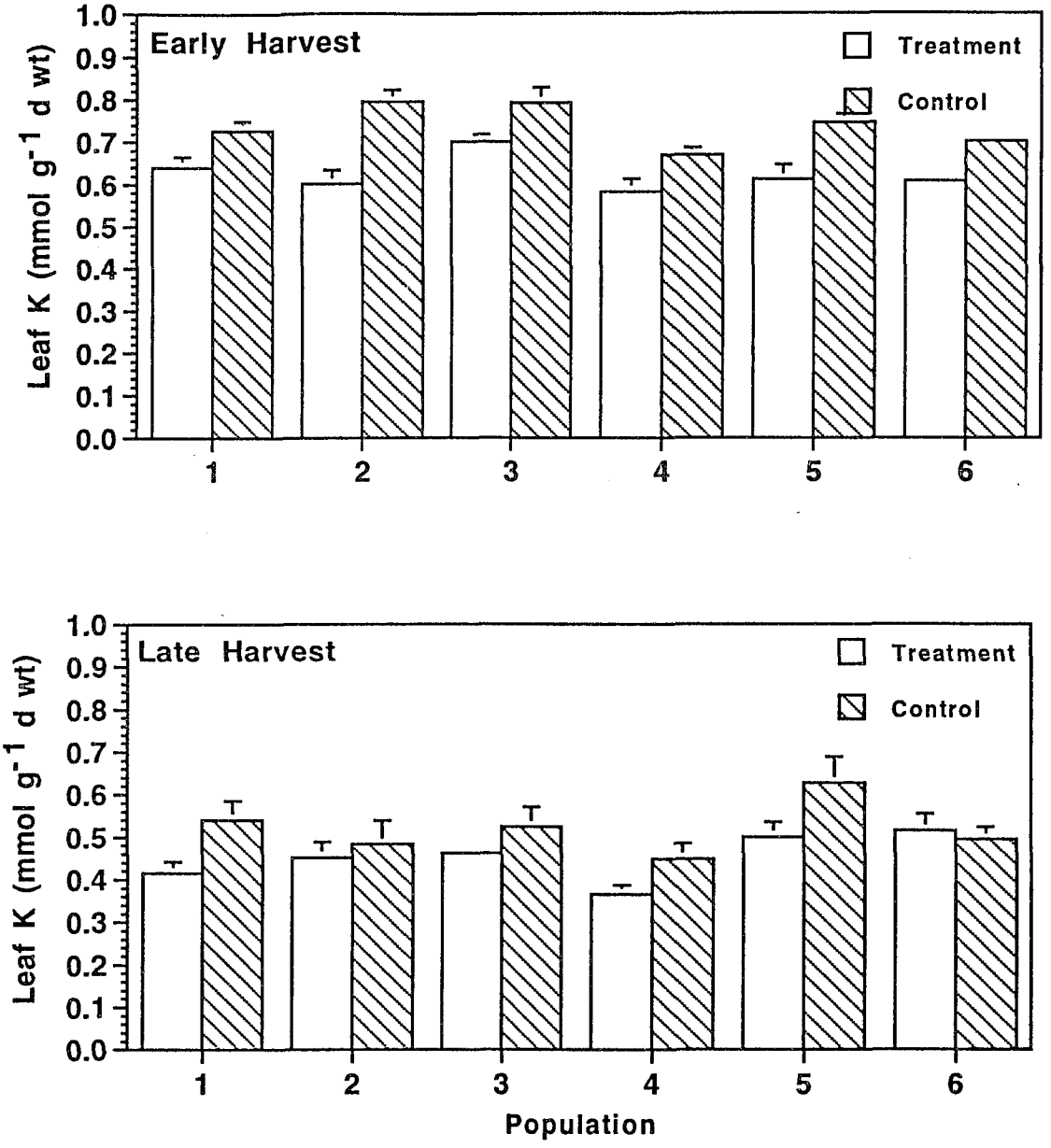

Figure 6.10. Mean (± std err) leaf K concentration (mmol g^{-1} d wt) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Spartina patens when subjected to a sublethal salinity excursion of 20‰ for one week (early harvest) and five weeks (late harvest); n=6, $LSD_{0.05}$=0.086, MSE=0.0057.

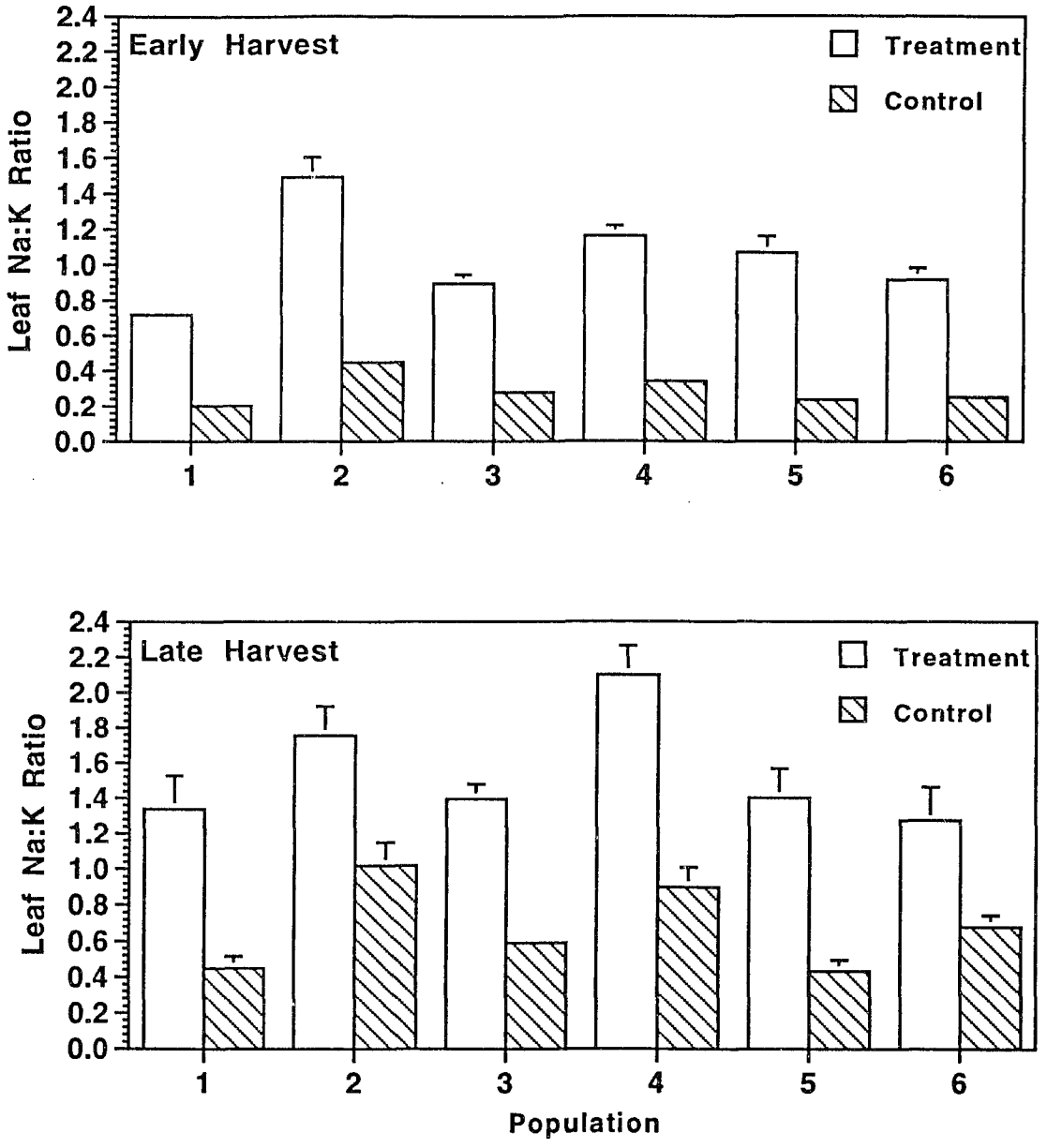

Figure 6.11. Mean (± std err) leaf Na:K ratio in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Spartina patens when subjected to a sublethal salinity excursion of 20‰ for one week (early harvest) and five weeks (late harvest); n=6, $LSD_{0.05}$=0.233, MSE=0.041.

salt-tolerant controls had greater leaf Ca than poorly salt-tolerant controls in both harvests, whereas contrasts within treatments showed that the highly salt-tolerant populations had significantly less leaf Ca than the poorly salt-tolerant populations, mainly due to the influence of high leaf Ca in population 5 (Table 6.1).

All main effects and interactions were significant for leaf Mg. There were no significant differences between highly salt-tolerant and poorly salt-tolerant populations within the controls in either harvest, nor within treatments in the early harvest. However, late harvest contrasts within treatments showed that the highly salt-tolerant populations had significantly less leaf Mg than poorly salt-tolerant populations (Table 6.2).

Leaf total cation concentrations displayed significant harvest, treatment and population main effects and a significant harvest x population interaction (Figure 6.12). Early harvest contrasts showed that there were no significant differences between highly salt-tolerant and poorly salt-tolerant populations. However, late harvest contrasts showed that although there were no significant differences between highly salt-tolerant and poorly salt-tolerant controls, contrasts within treatments showed that the highly salt-tolerant populations had significantly less total cation concentration than the poorly salt-tolerant populations (Figure 6.12). This difference was due primarily to the low total cation concentration of population 1, 1.055 mmol g^{-1} d wt, compared to a range of 1.210 to 1.334 mmol g^{-1} d wt for populations 2, 5, and 6 (Figure 6.12).

Leaf proline displayed a significant harvest main effect and a significant harvest x treatment interaction. Early harvest control proline levels were higher than expected and nearly as great as those within treatments. Contrasts within treatments detected that the poorly salt-tolerant populations had significantly greater proline levels than the highly salt tolerant populations in the early harvest only (Figure 6.13).

Leaf glycinebetaine levels displayed significant harvest and treatment main effects and a significant harvest x treatment interaction. There were no significant differences

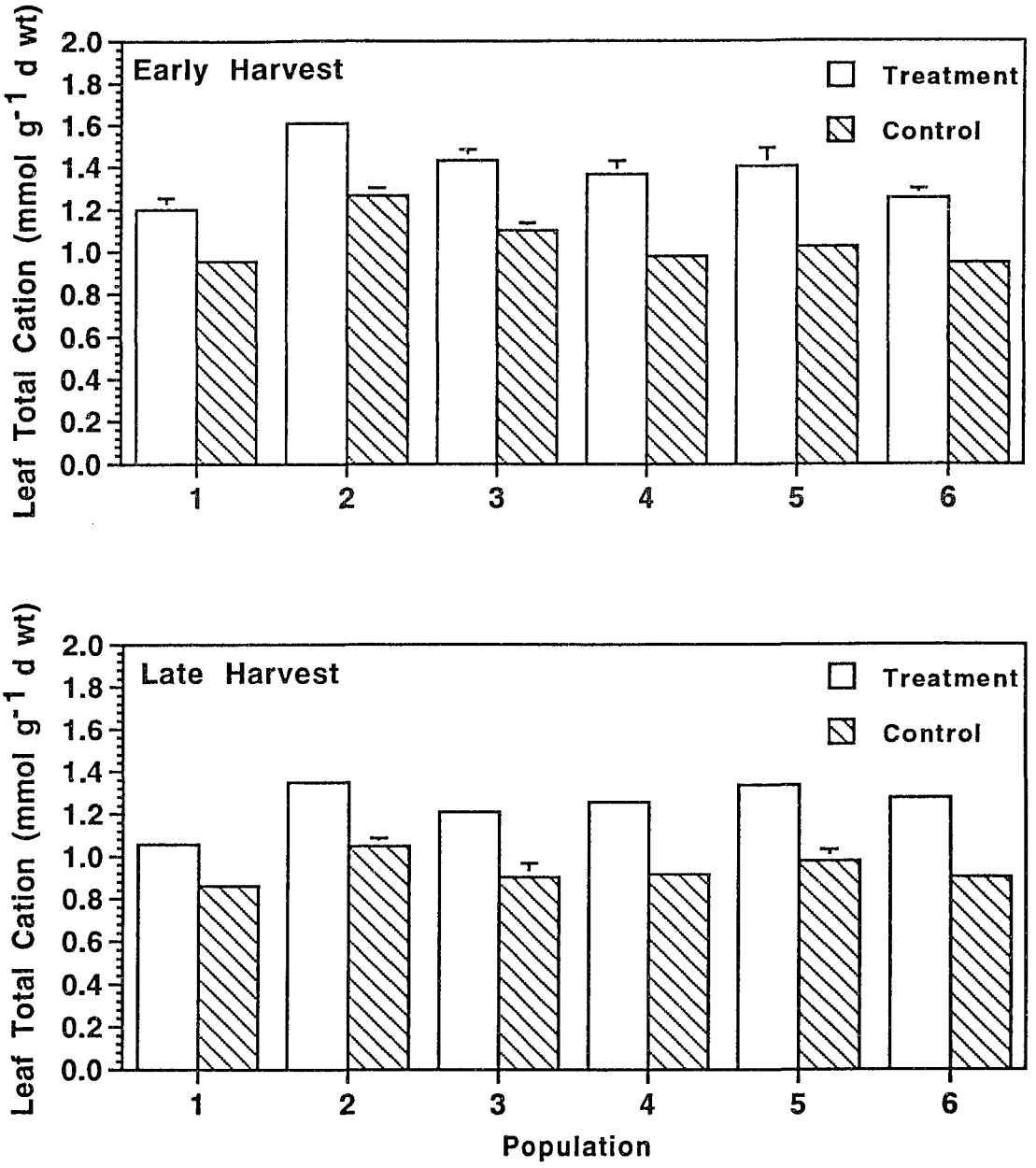

Figure 6.12. Mean (± std err) leaf total cation concentration (mmol g^{-1} d wt sum of Na, K, Ca, Mg) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Spartina patens when subjected to a sublethal salinity excursion of 20‰ for one week (early harvest) and five weeks (late harvest); n=6, $LSD_{0.05}$=0.101, MSE=0.0078.

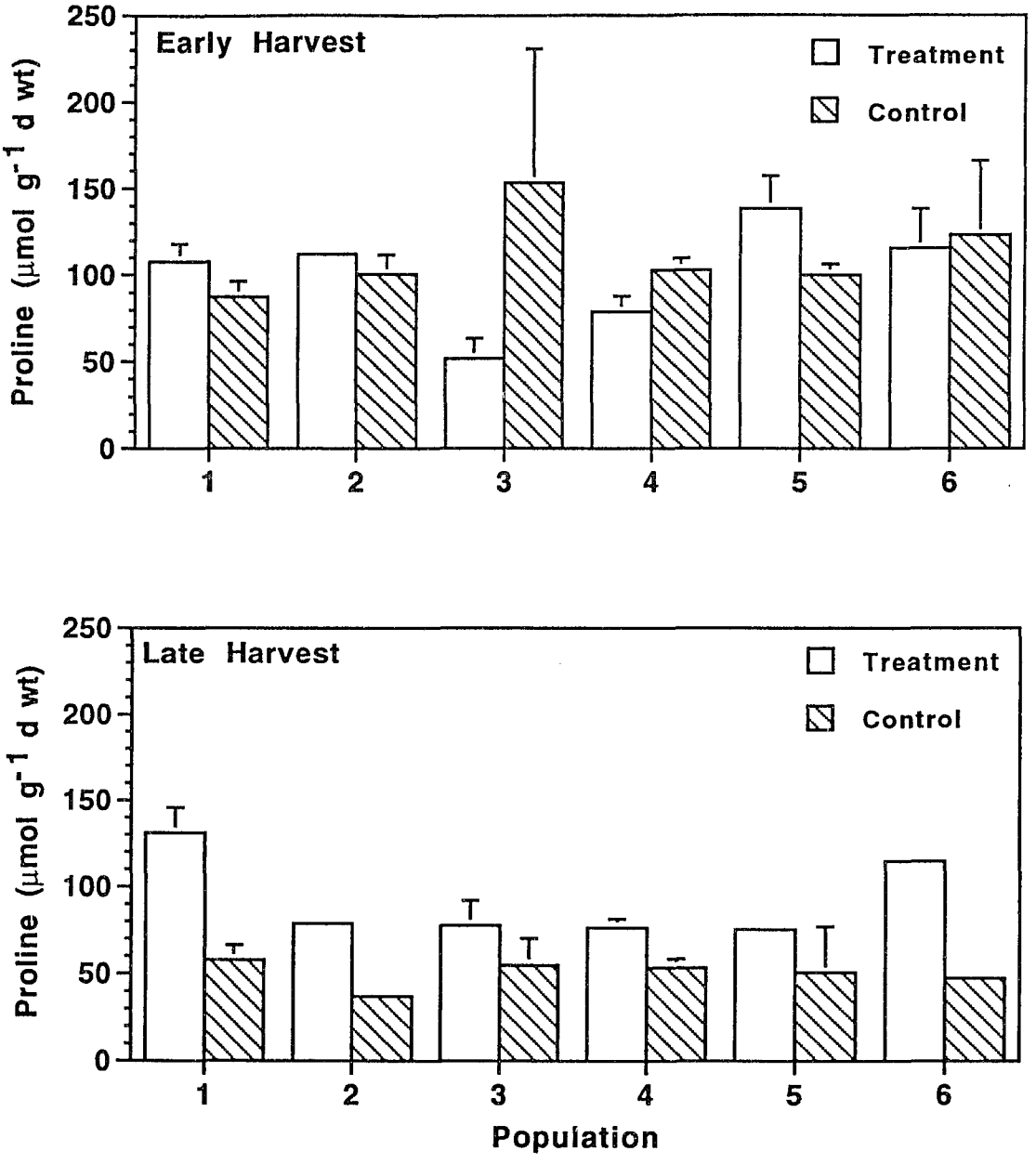

Figure 6.13. Mean (± std err) leaf proline concentration (µmol g^{-1} d wt) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Spartina patens when subjected to a sublethal salinity excursion of 20‰ for one week (early harvest) and five weeks (late harvest); n=3, $LSD_{0.05}$=61.56, MSE=1402.8.

with treatments between highly salt-tolerant and poorly salt-tolerant populations in either harvest (Figure 6.14).

Leaf sucrose in the salinity treatments was significantly elevated above the controls, but there was not a significant population effect (Figure 6.15). Similarly, contrasts between highly salt-tolerant and poorly salt-tolerant populations failed to detect any significant differences. Treatment sucrose concentrations average 33.4 μmol g^{-1} d wt compared to 22.3 μmol g^{-1} d wt for controls. There were no significant main effects or interactions detected in fructose, glucose or maltose. Respective mean treatment and control values were 1.53 and 1.98 μmol g^{-1} d wt for fructose, 1.34 and 0.07 μmol g^{-1} d wt for glucose and 0.03 and 0.45 μmol g^{-1} d wt for maltose.

Spartina alterniflora

Leaf xylem pressure displayed significant harvest and treatment main effects in addition to a significant treatment x population interaction. Early harvest leaf xylem pressures averaged -1.9 and -4.0 MPa for controls and treatments, respectively, compared to corresponding late harvest values of -1.4 and -3.5 MPa (Figure 6.16). There were no significant differences between highly salt-tolerant and poorly salt-tolerant populations within treatments or controls of either harvest.

Leaf Na displayed significant harvest, treatment and population main effects and significant harvest x treatment and harvest x population interactions. Contrasts showed that there were no significant differences between highly salt-tolerant and poorly salt-tolerant populations within treatments or controls of either harvest (Figure 6.17). However, contrasts within treatments showed that population 1 had significantly lower leaf Na than population 6 in both harvests (Figure 6.17), although it needs to be noted that population 5 (also classified as poorly salt tolerant) had leaf Na levels not significantly different from those of population 1 (Figure 6.17).

Leaf K showed significant harvest, treatment and population main effects. As was the case for Spartina patens, early harvest leaf K values (0.518 mmol g^{-1} d wt) were

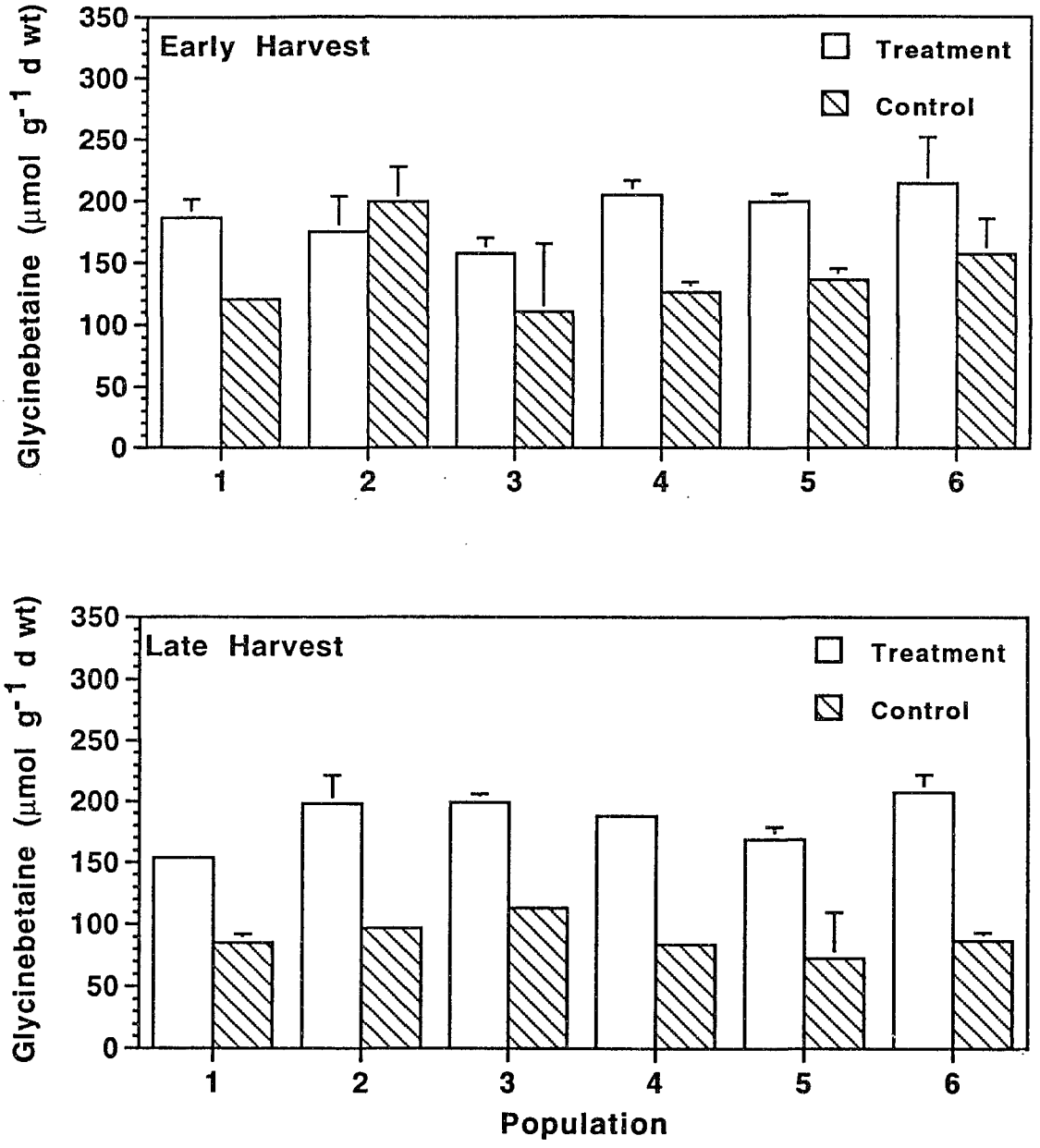

Figure 6.14. Mean (± std err) leaf glycinebetaine concentration (µmol g^{-1} d wt) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Spartina patens when subjected to a sublethal salinity excursion of 20‰ for one week (early harvest) and five weeks (late harvest); n=3; $LSD_{0.05}$=53.25, MSE=1049.6.

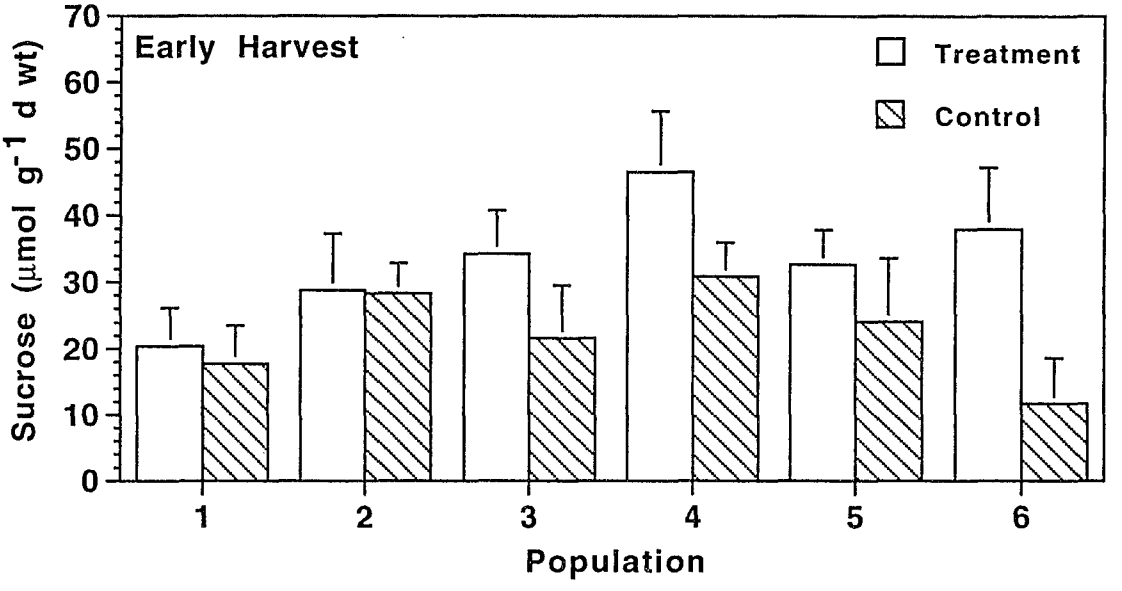

Figure 6.15. Mean (± std err) leaf sucrose concentration (µmol g^{-1} d wt) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Spartina patens when subjected to a sublethal salinity excursion of 20‰ for one week (early harvest); n=3, $LSD_{0.05}$=18.97, MSE=125.6.

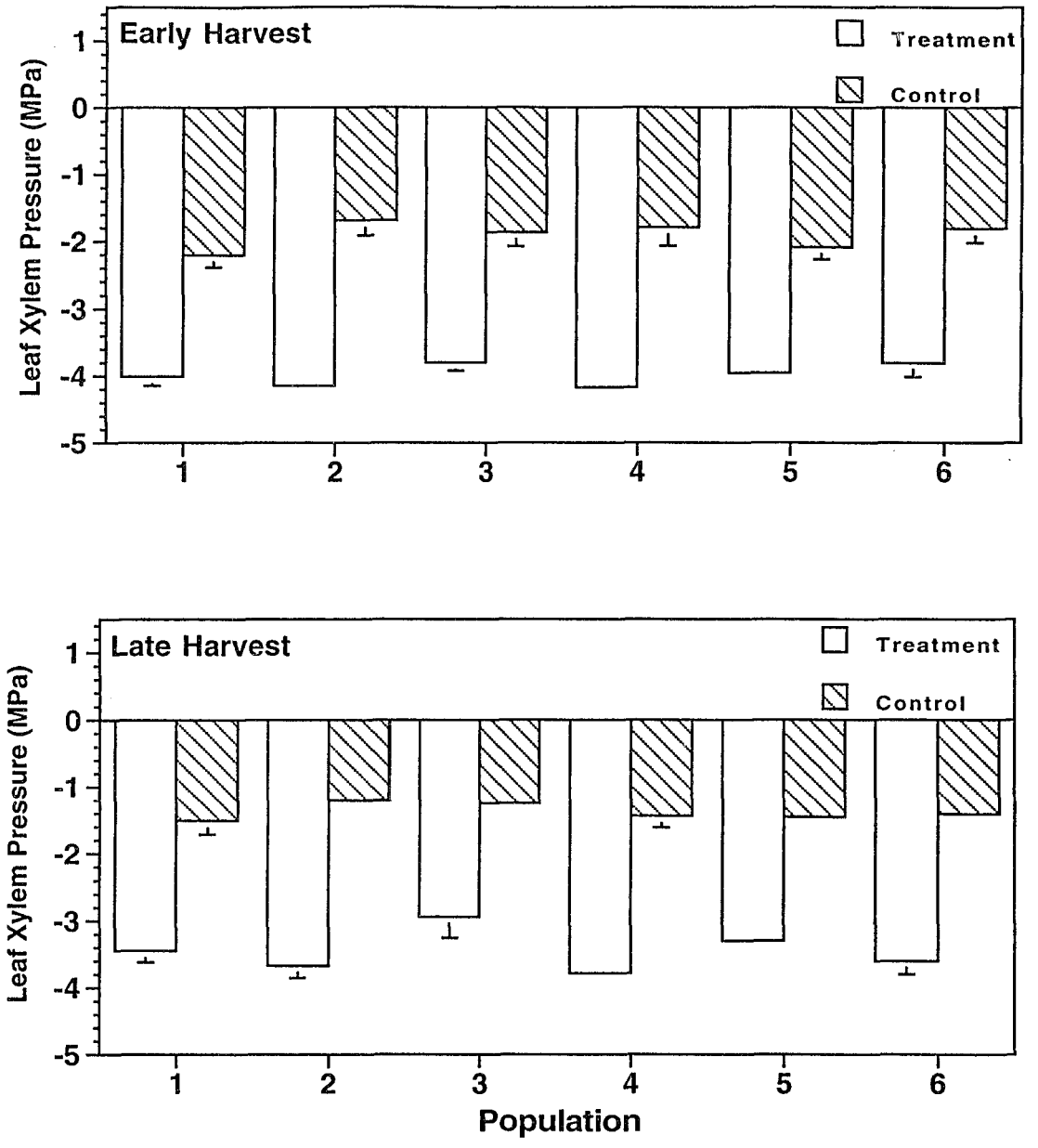

Figure 6.16. Mean (± std err) midday leaf xylem pressure (MPa) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Spartina alterniflora when subjected to a sublethal salinity excursion of 30‰ for one week (early harvest) and five weeks (late harvest); n=6, $LSD_{0.05}$=4.68, MSE=16.60.

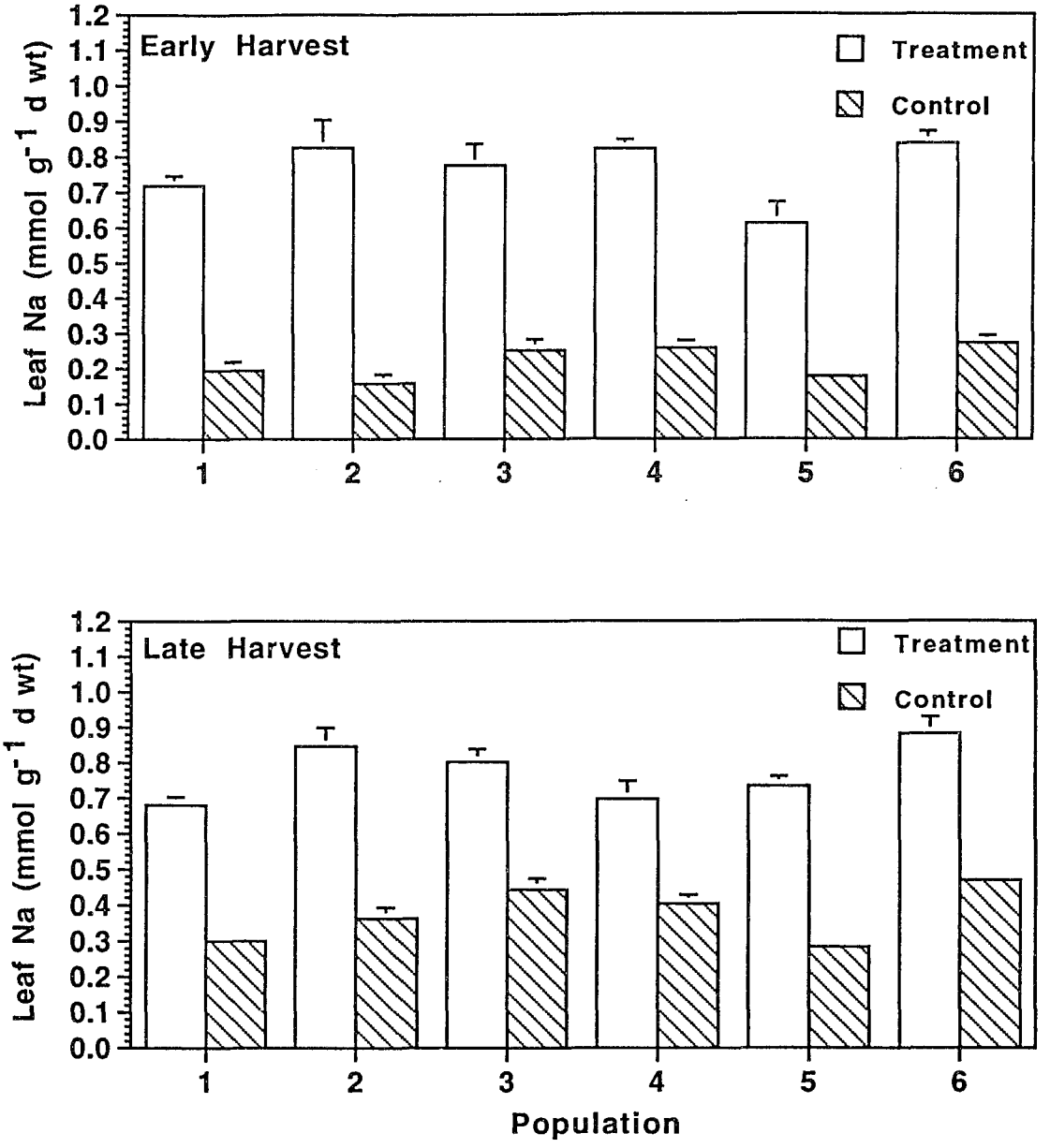

Figure 6.17. Mean (± std err) leaf Na concentration (mmol g^{-1} d wt) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Spartina alterniflora when subjected to a sublethal salinity excursion of 30‰ for one week (early harvest) and five weeks (late harvest); n=6, $LSD_{0.05}$=0.104, MSE=0.008.

greater than late harvest values (0.459 mmol g^{-1} d wt). Also, control leaf K values (0.580 mmol g^{-1} d wt) were greater than treatment values (0.400 mmol g^{-1} d wt; Figure 6.18). Contrasts showed no significant differences in the early harvest between highly salt-tolerant and poorly salt-tolerant populations within treatments or controls. However, in the late harvest the highly salt-tolerant populations had significantly greater leaf K (0.447 mmol g^{-1} d wt) than the poorly salt-tolerant populations (0.315 mmol g^{-1} d wt; Figure 6.18).

Sodium-to-potassium ratios in the leaf showed significant harvest, treatment and population main effects and all interactions with the exception of harvest x treatment (Figure 6.19). Contrasts within treatments in the late harvest showed that the highly salt-tolerant populations had significantly lower Na:K ratios (1.46 and 2.07 for populations 1 and 2, respectively) than the poorly salt-tolerant populations (2.53 and 2.63 for populations 5 and 6, respectively; Figure 6.19). Contrasts within controls showed no significant differences between highly salt-tolerant and poorly salt-tolerant populations in either harvest.

Leaf Ca and Mg both displayed significant harvest, treatment and population main effects and significant harvest x treatment and harvest x population interactions (Tables 6.1 and 6.2). Leaf Mg also had a significant treatment x population interaction. Within treatments, highly salt-tolerant populations had significantly greater leaf Ca and Mg than poorly salt-tolerant populations in the early harvest only. (Tables 6.1 and 6.2).

Leaf total cation concentrations displayed significant harvest, treatment and population main effects and a significant treatment x population interaction. Contrasts failed to detect any significant differences between highly salt-tolerant and poorly salt-tolerant populations within treatments or controls of either harvest (Figure 6.20).

Leaf proline displayed a significant treatment main effect and a significant harvest x treatment interaction. Contrasts within treatments failed to detect any significant differences between highly salt-tolerant and the poorly salt-tolerant populations in either

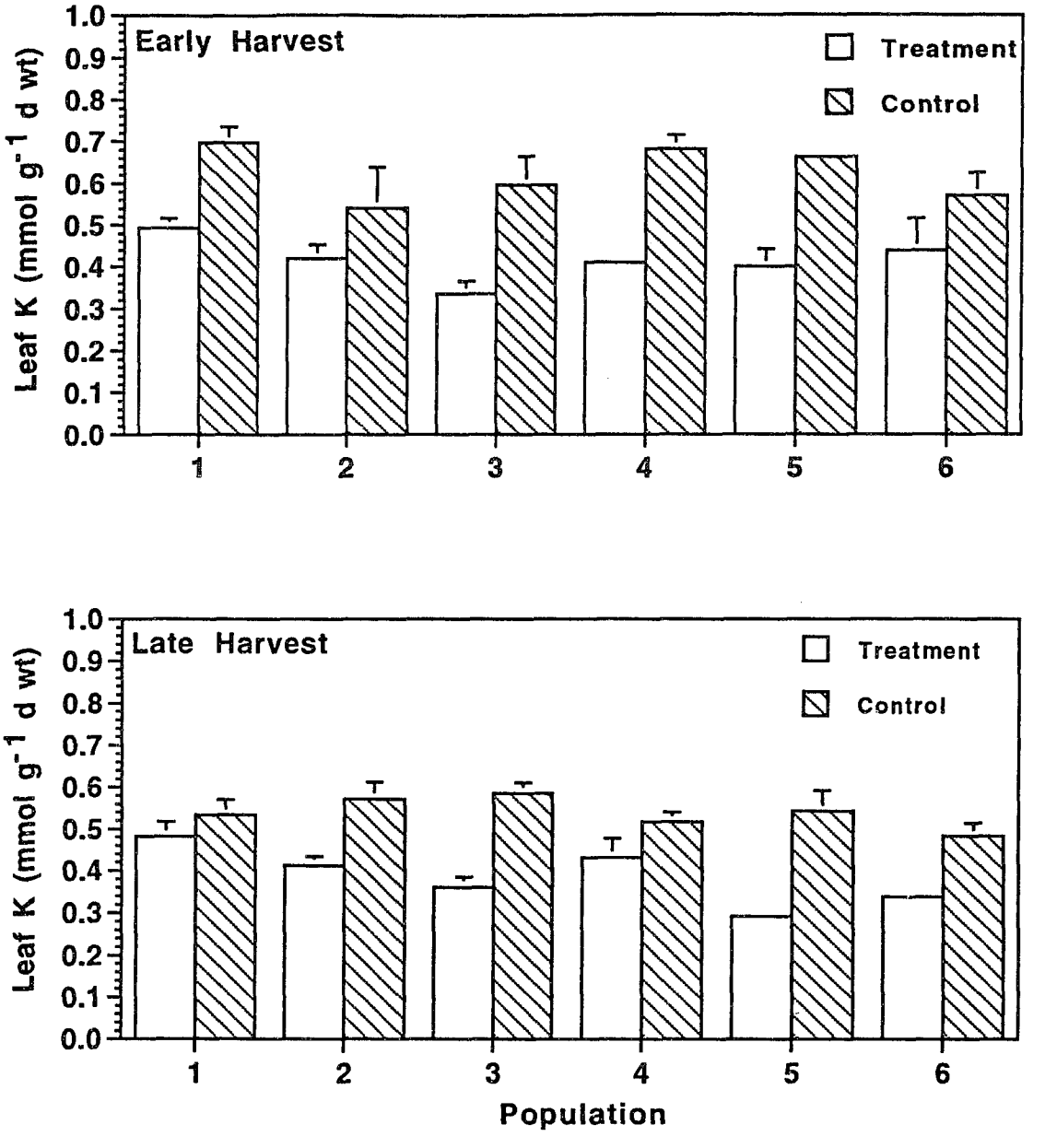

Figure 6.18. Mean (± std err) leaf K concentration (mmol g^{-1} d wt) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Spartina alterniflora when subjected to a sublethal salinity excursion of 30‰ for one week (early harvest) and five weeks (late harvest); n=6, $LSD_{0.05}$=0.116, MSE=0.010.

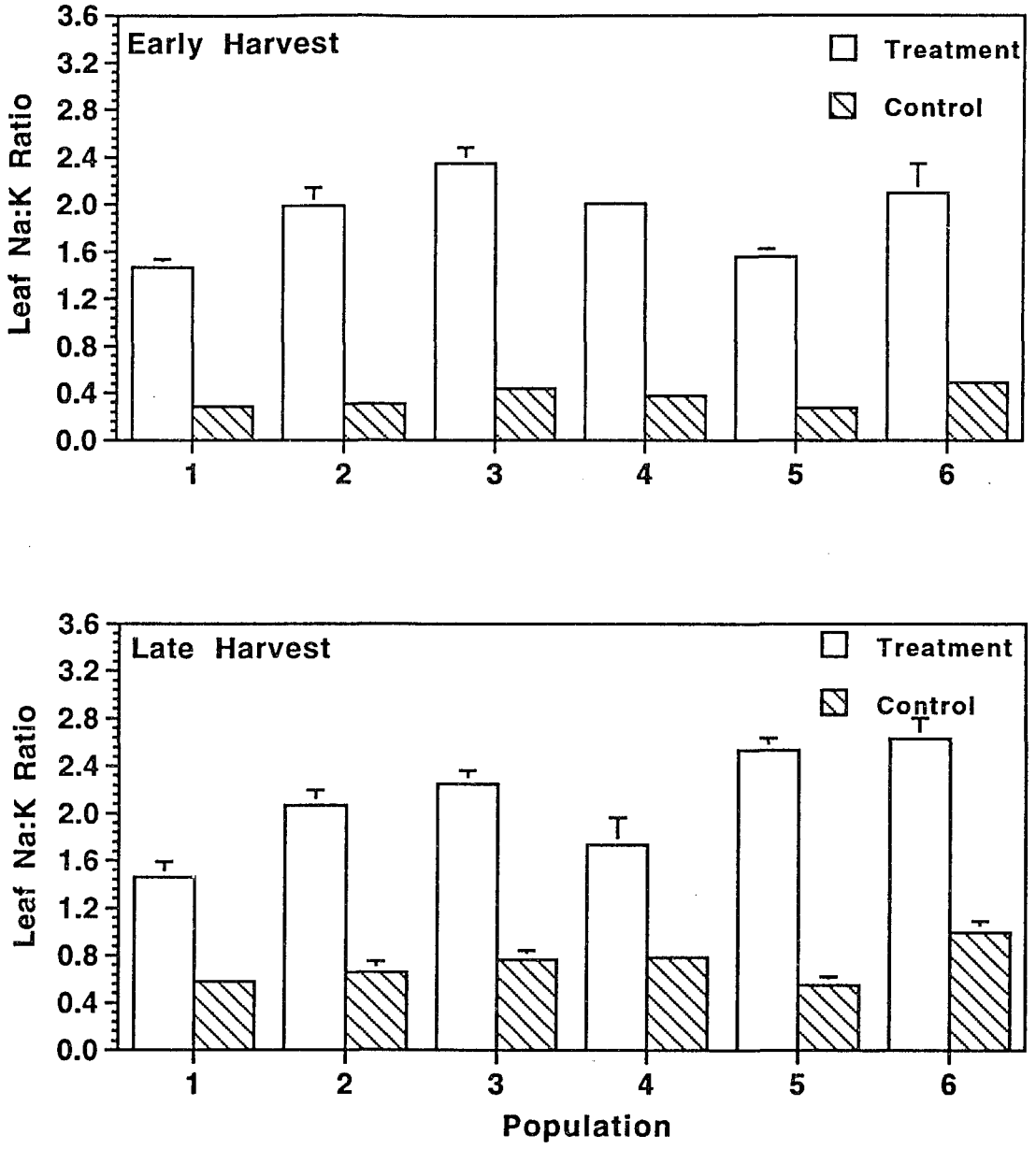

Figure 6.19. Mean (± std err) leaf Na:K ratio in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Spartina alterniflora when subjected to a sublethal salinity excursion of 30‰ for one week (early harvest) and five weeks (late harvest); n=6, $LSD_{0.05}$=0.318, MSE=0.075.

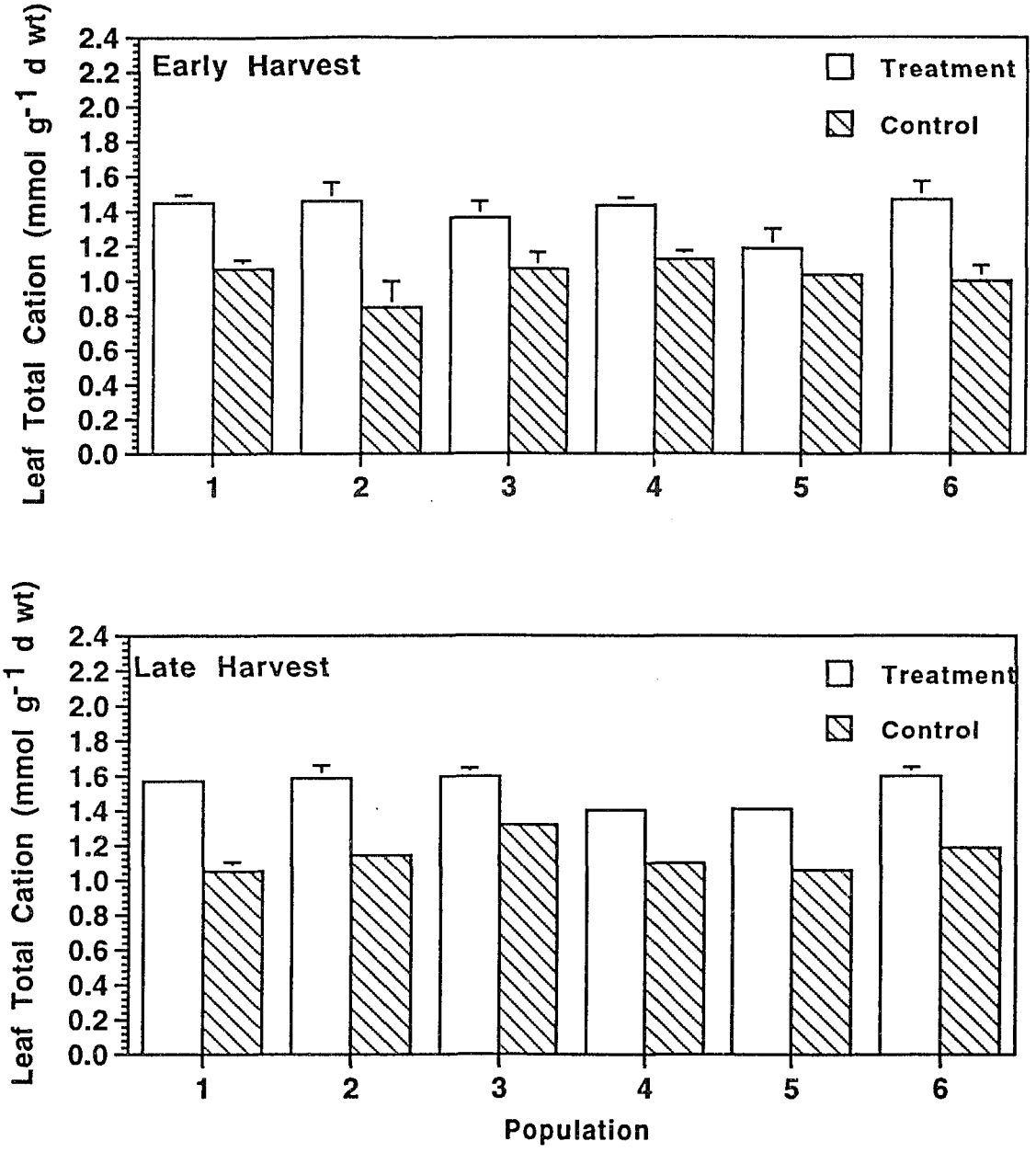

Figure 6.20. Mean (± std err) leaf total cation concentration (mmol g^{-1} d wt sum of Na, K, Ca, Mg) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Spartina alterniflora when subjected to a sublethal salinity excursion of 30‰ for one week (early harvest) and five weeks (late harvest); n=6, $LSD_{0.05}$=0.196, MSE=0.029.

harvest. In the early harvest, mean leaf proline concentrations were 16.7 μmol g^{-1} d wt and 53.5 μmol g^{-1} d wt for the controls and treatments, respectively, compared to respective control and treatment late harvest values of 22.9 μmol g^{-1} d wt and 40.9 μmol g^{-1} d wt (Figure 6.21).

Glycinebetaine showed a significant treatment main effect and significant harvest x treatment and harvest x population interactions. Contrasts within treatments showed that highly salt-tolerant populations had significantly greater glycinebetaine concentrations than poorly salt-tolerant populations during the early harvest only (Figure 6.22). This difference was primarily due to the relatively high glycinebetaine value of population 2 (203 μmol g^{-1} d wt) compared to that of population 5 (164 μmol g^{-1} d wt; Figure 6.22).

Leaf sucrose displayed a significant treatment effect with sucrose concentrations being elevated in the salinity treatments (Figure 6.23). There was not a significant population effect, nor any significant differences detected with contrasts between highly salt-tolerant and poorly salt-tolerant populations. Leaf sucrose averaged 22.7 μmol g^{-1} d wt in the salinity treatments compared to 16.4 μmol g^{-1} d wt in the controls. There were no significant main effects or interactions detected in leaf fructose, glucose and maltose. Respective mean control and treatment values were 7.50 and 7.66 μmol g^{-1} d wt for fructose, 0.57 and 0.30 μmol g^{-1} d wt for glucose, and 0.003 and 0.07 μmol g^{-1} d wt for maltose.

DISCUSSION

Leaf xylem pressures in the poorly salt-tolerant populations of Panicum hemitomon were significantly more negative than those of the highly salt-tolerant populations during the early harvest, but not during the late harvest. The less negative leaf xylem pressure of the highly salt-tolerant populations may be associated with less salt uptake during the first week of exposure to elevated salinity. Many glycophytes are able to respond to moderate levels of salinity by excluding sodium and chloride as a

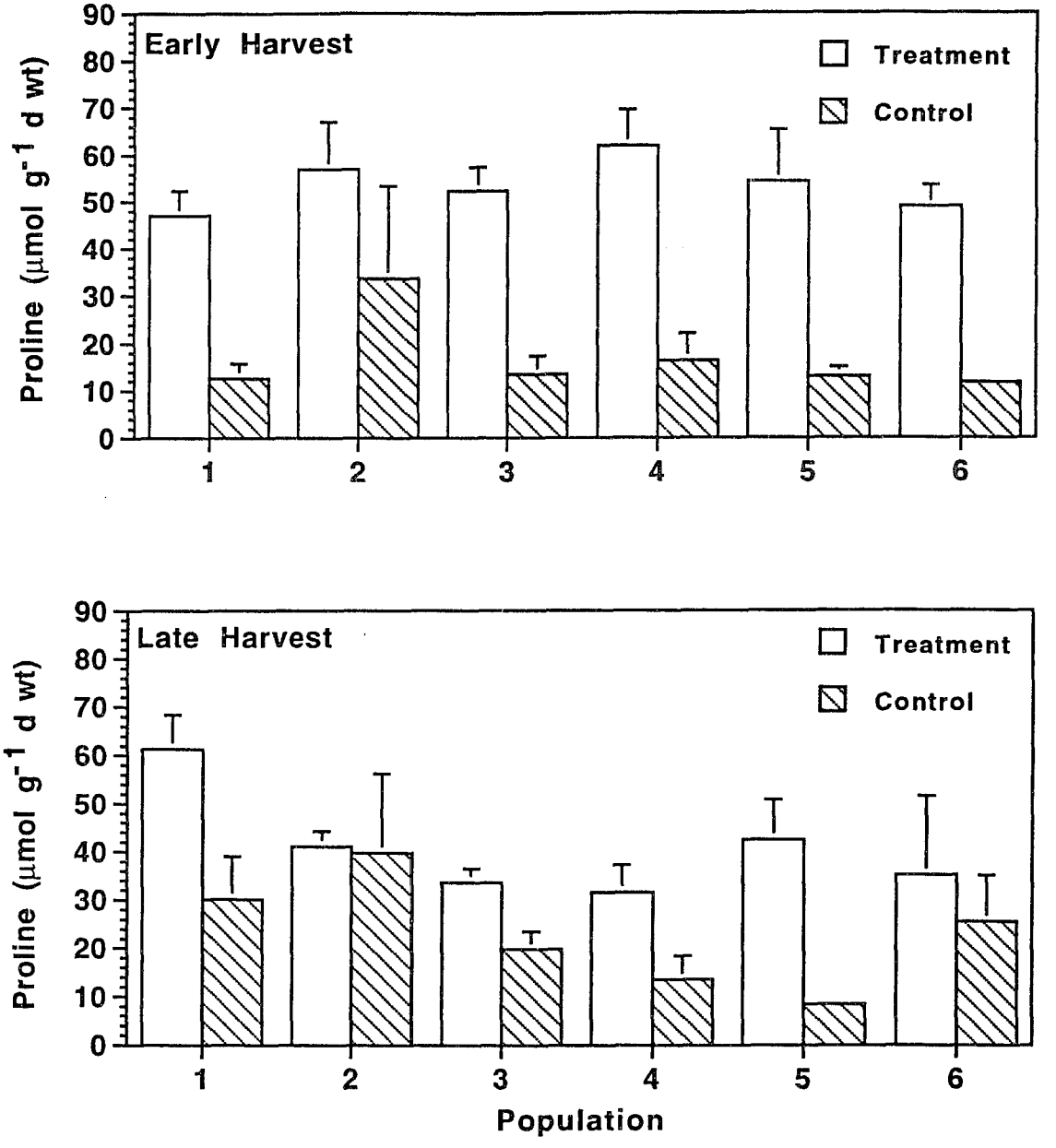

Figure 6.21. Mean (± std err) leaf proline concentration (µmol g^{-1} d wt) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Spartina alterniflora when subjected to a sublethal salinity excursion of 30‰ for one week (early harvest) and five weeks (late harvest); n=3, $LSD_{0.05}$=22.57, MSE=188.6.

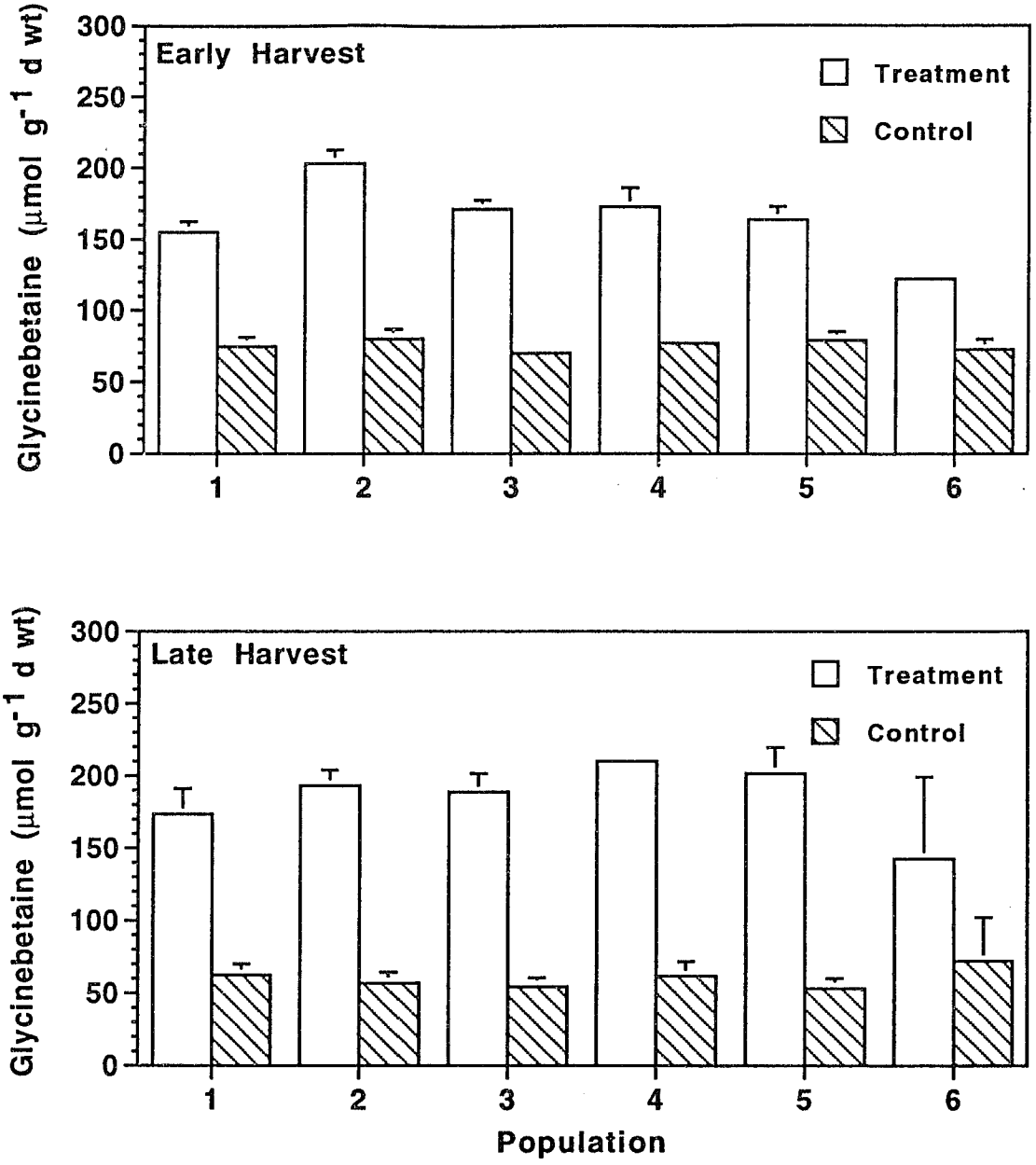

Figure 6.22. Mean (± std err) leaf glycinebetaine concentration (µmol g^{-1} d wt) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Spartina alterniflora when subjected to a sublethal salinity excursion of 30‰ for one week (early harvest) and five weeks (late harvest); n=3, $LSD_{0.05}$=44.86, MSE=744.9.

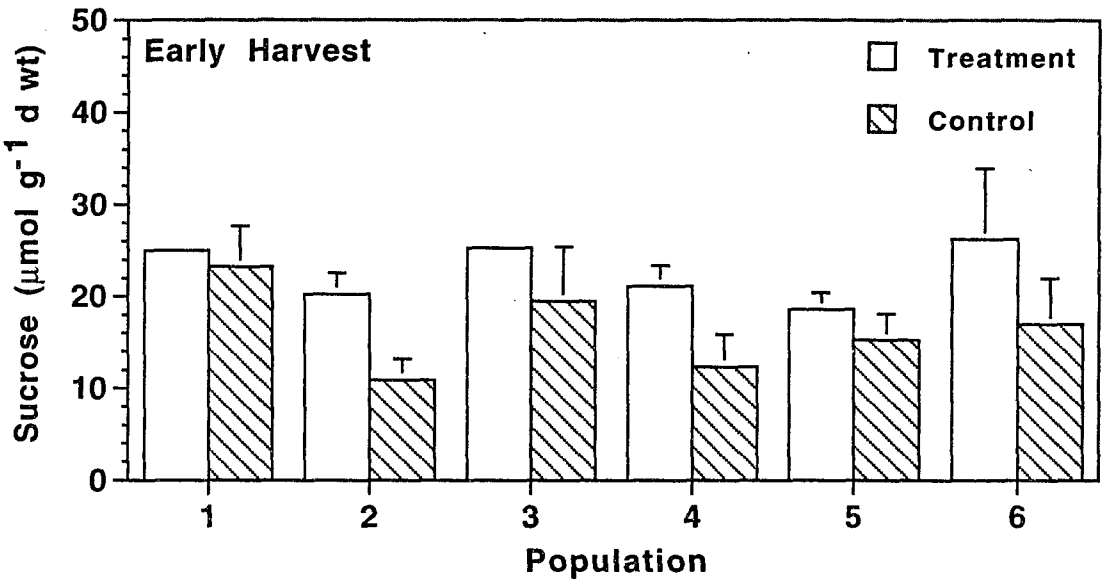

Figure 6.23. Mean (± std err) leaf sucrose concentration (µmol g^{-1} d wt) in treatments and controls of highly salt-tolerant (populations 1 and 2), intermediate salt-tolerant (populations 3 and 4) and poorly salt-tolerant (populations 5 and 6) populations of Spartina alterniflora when subjected to a sublethal salinity excursion of 30‰ for one week (early harvest); n=3, $LSD_{0.05}$=11.60, MSE=46.95.

mechanism of tolerance (Sacher et al. 1982), although accumulation of sodium and chloride in salt-adapted glycophyte cells in tissue culture and whole plants has also been observed (Greenway and Munns 1980; Binzel et al. 1987; Cheeseman 1988). In this study, Panicum leaf Na levels in the salinity treatments were typically three to four times greater than those in the controls. McKee and Mendelssohn (1989) reported Panicum hemitomon grown at different salinities had little control over Na uptake, since leaf Na was positively correlated with interstitial salinity. Contrasts within early harvest treatments showed that leaf total cation concentration was significantly greater in the poorly salt-tolerant populations, thereby indicating greater ion uptake by the poorly salt-tolerant populations during the initial week of exposure to elevated salinities. This agrees with the generality that an inverse relationship typically exists between salt uptake and salinity resistance in closely related varieties or species of glycophytes (Greenway and Munns 1980; Yeo 1983). In this study, population 1 (highly salt-tolerant) displayed a significantly greater contribution of Ca to total leaf cation concentration than observed in population 6 (poorly salt-tolerant), possibly indicating some selectivity in relative ion contribution to total cation concentration in this more highly salt-tolerant population (Greenway and Munns 1980; Cramer et al 1986).

Early harvest Panicum leaf proline was also significantly elevated in the poorly salt-tolerant populations and confirms that these less salt-tolerant populations were experiencing earlier and more severe salt-stress than the highly salt-tolerant populations, since proline generally accumulates to significant levels only after a certain threshold of salinity or water stress is exceeded (Cavalieri and Huang 1979). In the late harvest, Panicum proline remained significantly elevated in population 6 of the poorly salt-tolerant populations compared to the highly salt-tolerant populations (recall that by the late harvest none of the experimental units in population 5 had sufficient green tissue for analysis).

Panicum hemitomon apparently has only a limited amount of control over ion uptake in terms of selectivity or exclusion, since by the late harvest both highly salt-tolerant and poorly salt-tolerant populations showed signs of cumulative salt stress with observed tissue necrosis and very high leaf cation concentrations. Previous results of these investigations (Chapters 2 and 5) consistently show plant size to be important in mitigating the severity of salt stress in Panicum hemitomon, with the more salt-tolerant populations achieving larger size (greater biomass) than the poorly salt-tolerant populations. Larger size results in more mature tissue available for the translocation of ions away from actively growing regions (Fitter and Hay 1987; Munns 1993). The results from Chapter 5 confirmed greater aboveground biomass in the highly salt-tolerant populations and a larger proportion of dead aboveground biomass in the poorly salt-tolerant populations.

Halophytes, are able to accumulate fairly high concentrations of salts in their tissues for osmotic adjustment through compartmentalization of ions in vacuoles and the production of neutral organic solutes in the cytoplasm (Flowers 1985; Gorham et al. 1985). Leaf cation concentrations in Spartina patens and Spartina alterniflora were significantly elevated in the salinity treatments relative to their respective controls. In the Spartina patens salinity treatments, late harvest total leaf cation concentrations were significantly greater in the poorly salt-tolerant populations and leaf xylem pressures more negative ($P \leq 0.10$) compared to the highly salt-tolerant populations.

Early harvest proline accumulation also indicated that the poorly salt-tolerant Spartina patens populations were experiencing a more severe salt/water stress than the highly salt-tolerant populations. The relatively high proline levels in controls were unexpected and may reflect a residual water stress response from handling and separating tillers during transplanting, since proline levels decreased by the late harvest. Some of the other differences between highly salt-tolerant and poorly salt-tolerant populations in Spartina patens were limited to comparisons between population 1 and population 6,

such as leaf Na . Population 2 had a tendency to accumulate fairly high levels of Na, both in controls as well as treatments compared to population 1, which had significantly lower leaf Na than population 6 in both harvests, possibly indicating that this population may be more efficient at selectively excluding or secreting Na (Smart and Barko 1980; Bradley and Morris 1991). Some of the variation between populations of known similar lethal salinity tolerance (i.e. populations 1 and 2) may be due to different mechanisms of salt tolerance operating at different intensities (Yeo et al. 1990).

Spartina alterniflora was unlike Panicum and Spartina patens in that it did not display significant differences in leaf xylem pressure and total cation concentrations between highly and poorly salt-tolerant populations. More importantly, Spartina alterniflora did display some ion selectivity responses with highly salt-tolerant populations tending to have greater leaf K levels than the poorly salt-tolerant populations by the late harvest and also significantly lower leaf Na concentrations (population 1 vs. Population 6 in the early harvest). Leaf Na:K ratios were also significantly less in the highly salt-tolerant populations by the late harvest, whereas controls displayed no significant differences. It is well-documented that Spartina alterniflora can exert fairly dramatic control over ion accumulation via selective processes of ion exclusion and secretion (Smart and Barko 1980), particularly in terms of selective exclusion and secretion of Na^+ over K^+ (Bradley and Morris 1991).

Ion exclusion is believed to be the predominant mechanism controlling ion accumulation in Spartina alterniflora, and may account for excluding greater than 90% of the theoretical maximum ion uptake that would result from transpiration and growth (Bradley and Morris 1991). Furthermore, salt glands in the leaves of Spartina alterniflora are capable of secreting approximately half of the ions that are taken up, although this process may be less ion specific (Bradley and Morris 1991). Since aboveground biomass in this study did not differ significantly between populations (previous chapter), it would appear that population differences in exclusion and secretion

pathways are largely responsible for the observed differences in tissue ion concentrations, not differences in growth rates which may affect tissue concentrations via ion dilution effects (Yeo et al. 1990).

Population differences in Spartina alterniflora leaf calcium and magnesium concentrations were also observed in the early harvest, but it is unclear whether the magnitude of these differences in Ca concentration would confer a salt-tolerance advantage via maintenance of plasma membrane integrity (Hanson 1984; Blits and Gallagher 1990b) which has been shown to be required for functional K/Na selectivity (Epstein 1961; Lauchli and Epstein 1970; Cramer et al. 1985).

Increases in sucrose concentration and other soluble sugars under salinity stress have been reported in glycophytes and halophytes (Greenway and Munns 1980; Briens and Larher 1982; Flowers 1985; Binzel et al. 1987; Cheeseman 1988). All species showed an increase in leaf sucrose levels with sublethal salinity stress, but not in a manner related to known salinity tolerance. Although the soluble sugar levels in this study were generally less than reported by Briens and Larher (1982), our results are consistent with their finding that sucrose was the predominant soluble sugar in the many halophytic species they investigated, and that glucose accumulated to only a very limited extent in halophytes. Although not statistically significant, in this study it appeared that there was a tendency for the highly salt-tolerant populations of Spartina patens to display lower sucrose levels under salt stress than the poorly salt-tolerant populations, as well as exhibit less of a stimulation in sucrose accumulation above controls upon exposure to sublethal salinity levels.

In summary, all three species exhibited more negative leaf xylem pressures and greater leaf cation concentrations under sublethal salinity stress than the controls. Differences in response to salinity between highly salt-tolerant and poorly salt-tolerant populations varied depending on the species. In Panicum hemitomon and Spartina patens the highly salt-tolerant populations displayed greater leaf xylem pressures and

lower leaf total cation concentrations than the poorly salt-tolerant populations, whereas in Spartina alterniflora there were no significant population differences in these variables. Salt stress resulted in significantly greater leaf proline levels in poorly salt-tolerant populations of Panicum hemitomon in both harvests and in poorly salt-tolerant populations of Spartina patens in the early harvest only. Although Spartina alterniflora leaf proline levels were significantly elevated by the salinity excursion, differences between populations were not evident. However, Spartina alterniflora glycinebetaine levels were greater in highly salt-tolerant populations during the early harvest. The more salt-tolerant populations of Panicum hemitomon and Spartina patens were able to limit leaf cation concentrations to some extent, possibly via ion exclusion and greater shoot growth, and in the case of Spartina patens, possibly also due to greater salt secretion. Spartina alterniflora population differences were not evident in leaf total cation concentrations, but highly salt-tolerant populations displayed more control over ion selectivity than the poorly salt-tolerant populations by accumulating more Ca and Mg in the early harvest and proportionately more K than Na by the late harvest.

Chapter 7

Conclusions

All three of the plant species investigated in this study displayed significant intraspecific variation in salt tolerance and plant morphology. Lethal salinity levels of plant populations allowed to de-acclimate from field salinity conditions for several vegetative generations ranged from 7.6‰ to 12.0‰ in Panicum hemitomon, from 63‰ to 93‰ in Spartina patens, and from 83‰ to 115‰ in Spartina alterniflora, representing more than an order of magnitude difference in lethal salinity levels between the fresh marsh dominant, Panicum hemitomon and the salt marsh dominant, Spartina alterniflora. The lethal salinity levels of Spartina patens were surprisingly high and overlapped with those of Spartina alterniflora. Although the weekly stepwise salinity increments were different for each species, thereby making species comparisons in lethal salinity levels tenuous, the results do illustrate the disparity in salt tolerance in Panicum hemitomon relative to those observed in the two Spartinas, and coincide with the distributions of these species in the field.

The distribution of Panicum hemitomon in the field is limited to fresh water areas, whereas Spartina alterniflora is well known for its dominance in coastal salt marshes. Spartina patens is unique, in that it has a very wide ecological amplitude ranging from fresh to intermediate marshes, where its distribution may overlap with Panicum, to brackish marshes, where it is the dominant emergent macrophyte and then overlaps distribution with Spartina alterniflora in brackish/saline marsh zones. Spartina patens is also a conspicuous component of barrier island dune and swale plant communities, where drought tolerance becomes an additional plant trait critical to survival. The ability of Spartina patens to tolerate drought stress in addition to salt stress allows this species to grow under a wide range of salt and water stress conditions in the field, and may have

contributed to some of the spurious results observed for this species in the sublethal salinity stress experiments.

Species can be classified in terms of their salt tolerance as glycophytes or halophytes, with halophytes being further subdivided into facultative halophytes or obligate halophytes (Sharma and Gupta 1986; Hale and Orcutt 1987), although even finer divisions have been suggested by Chapman (1966) and others. Based on this classification scheme, Panicum hemitomon may be defined as a glycophyte since its distribution is limited to non-saline areas, Spartina patens as a facultative halophyte since it may occur in environments ranging from fresh to saline, and Spartina alterniflora also as a facultative halophyte, but with its distribution generally restricted to coastal salt marshes. Results from investigations of plant morphology showed that although all three species displayed significant population differences in plant morphology, morphological traits were most associated with salt tolerance in the glycophyte, Panicum hemitomon, somewhat associated with salt tolerance in the facultative halophyte with a wide ecological amplitude, Spartina patens, and not associated with salt tolerance in the facultative halophyte, whose distribution is generally restricted to coastal salt marshes, Spartina alterniflora.

In Panicum, plant morphology characteristics associated with plant size, particularly leaf length and leaf length x width, were able to explain a considerable amount of the population variation in salt tolerance. Leaf length in Spartina patens was associated with population salt tolerance, but not as strongly as observed in Panicum. None of the morphological variables of the Spartina alterniflora populations in this study showed any association with salt tolerance, although highly salt-tolerant populations displayed less of a leaf rolling response at sublethal salinity levels. These results suggest that in Panicum, since it has only limited physiological control over salt stress, plant size factors are important in providing more tissue for the translocation of salts away from actively growing regions (Yeo et al. 1990; Munns 1993). In the more salt-tolerant species,

Spartina patens and Spartina alterniflora, some factors other than plant morphology become increasingly important in explaining the observed intraspecific variation in salt tolerance.

Investigations conducted at sublethal salinity levels within each of the three species revealed some interesting similarities and differences in physiological, biochemical and growth responses. Plant photosynthetic response (net CO_2 assimilation) was able to differentiate highly salt-tolerant and poorly salt-tolerant populations within each species to varying degrees; the differences being greatest in Panicum populations, with the highly salt-tolerant populations having higher photosynthetic rates. Highly salt-tolerant populations of Panicum hemitomon also initially displayed significantly greater plant water use efficiencies under salt stress than poorly salt-tolerant populations, whereas water use efficiencies did not differ significantly between populations of either of the Spartinas.

Population growth responses at sublethal salinity levels showed that the highly salt-tolerant populations of Panicum produced greater total biomass and had a greater proportion of live aboveground biomass than the poorly salt-tolerant populations. Biomass differences between populations of varying salt tolerance were less significant in Spartina patens and lacking in Spartina alterniflora, although the proportion of live aboveground biomass was greater in the more salt-tolerant Spartina alterniflora populations.

In Panicum hemitomon and Spartina patens, the highly salt-tolerant populations displayed greater initial leaf xylem pressures and lower leaf total cation concentrations than the poorly salt-tolerant populations, whereas in Spartina alterniflora there were no population differences in these variables. However, Spartina alterniflora displayed a greater ion selectivity response than either Spartina patens or Panicum hemitomon. In Spartina alterniflora, although total cation concentrations did not differ between

populations, the highly salt-tolerant populations were able to maintain lower Na:K ratios than the poorly salt-tolerant populations.

Investigations of the stress metabolite proline, which accumulates only after a certain threshold of salt or water stress is exceeded (Cavalieri and Huang 1979; 1981), yielded results that generally supported the other findings from the sublethal salinity stress experiments. Poorly salt-tolerant populations of Panicum hemitomon had significantly greater proline levels in both harvests, compared to Spartina patens, in which proline levels in poorly salt-tolerant populations were significantly elevated only in the early harvest. In Spartina alterniflora, there were no population differences in proline, but glycinebetaine, a compatible organic solute that may function in osmotic adjustment under salinity and water stress (Ladyman et al. 1980; Cavalieri and Huang 1981), was greater in the highly salt-tolerant populations during the early harvest.

Species and population differences in salt exclusion and salt secretion were not directly investigated in this study and may show promise for future research. Highly salt-tolerant populations of Panicum hemitomon were able to achieve significantly lower total leaf cation concentrations than the poorly salt-tolerant populations, but the relative contribution of ion exclusion, which is observed in some glycophytes (Binzel et al. 1987), versus greater aboveground growth, which can essentially dilute tissue ion concentrations (Yeo et al. 1990; Munns 1993), cannot be deciphered from the present study. Similarly, in Spartina patens and Spartina alterniflora, further research on the relative importance of salt secretion, salt exclusion, and growth differences in modifying tissue ion concentrations may be useful in further explaining the observed patterns of salt tolerance in the different populations of this study. It is not unreasonable to assume that different populations may achieve similar levels of salt tolerance through different relative contributions of salt tolerance traits or mechanisms, as has been reported in rice (Yeo et al. 1990).

A very important finding of this research is that within each of these dominant coastal grass species, significant intraspecific variation in salt tolerance was identified. There are two important consequences that stem from this finding. First, the identification of intraspecific variation in salt tolerance within these species allows for the selection of superior salt-tolerant planting stock that may be utilized in marsh creation or marsh restoration projects. The experiments conducted in this study have identified population differentiation in lethal salinity level in Gulf Coast populations of Panicum hemitomon, Spartina patens and Spartina alterniflora, as well as investigated the potential of certain plant traits and responses as markers of salinity tolerance that may facilitate future screenings. Furthermore, the results of the Spartina patens salinity screenings indicate that one cannot assume that a population established in an area of relatively higher field salinity will necessarily display higher salt tolerance than a population that has become established in an area of lower salinity once de-acclimated from the field. A logical extension of this work would be to conduct similar investigations on a different set of populations to confirm and expand upon the results of this study, as well as to conduct additional studies on the interactive effects of salinity and waterlogging. Since increases in salinity regime have been implicated as a factor in wetland loss, the technology to select plant populations best suited to meet the needs of various marsh restoration/creation sites is valuable and timely.

A second consequence of this research is that the vast expanses of monospecific stands of these species often observed in coastal marshes are, in fact, probably not as uniform as they appear to the eye. A tremendous amount of effort has been invested in research to investigate underlying causes of marsh loss and marsh dieback in Louisiana. Although it cannot be argued that abiotic factors such as salinity and depth and duration of flooding are important, population differences in response to these stresses may be an important modifying factor that has been largely overlooked. Future research on the relative contribution of genotypic differences between plant populations, regarding their

stress response and clonal structure, to the observed patterns of marsh dieback may prove rewarding.

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VITA

Mark Warner Hester was born in Hammond, Indiana, on January 15, 1958. Mark acquired an appreciation of nature at an early age from his parents and from his family's frequent trips to their cabin on inland lake in Michigan. By the age of five Mark was an avid fisherman and had already expressed interest in a career in one of the life sciences. Mark attended Hammond Morton Senior High School, where he was co-valedictorian of the class of 1976. Mark pursued his interest in Biology and in 1980 received the Degree of Bachelor of Arts in Biology with honors from Indiana University.

During his last year at Indiana University, Dr. Irving A. Mendelssohn, then an assistant professor at Louisiana State University, phoned Mark and expressed interest in having Mark work under him as Dr. Mendelssohn's first Masters student on a project investigating factors that could be limiting the distribution of sea oats along the Louisiana coast. That fall, Mark began his graduate research in the Department of Marine Sciences at Louisiana State University under the guidance of Irv Mendelssohn, and has been involved in coastal plant ecophysiological research ever since. In 1983 Mark began working as a full-time research associate for Dr. Mendelssohn. Mark received his Masters degree in Marine Sciences in 1985.

In 1986 Mark married his beloved wife, Mary, who had received her Masters in English at Louisiana State University. Mark continued his association with Dr. Mendelssohn, and after a few years, with the encouragement and support of his wife and Dr. Mendelssohn, Mark decided to pursue a part-time Ph.D. while continuing full-time employment. Mark completed his Ph.D. in Oceanography and Coastal Sciences with minors in Botany and Experimental Statistics in 1995. Mark has accepted a tenure-track assistant professor position in the Department of Biological Sciences at Southeastern Louisiana University, where he will begin employment in August 1995.

DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Mark Warner Hester

Major Field: Oceanography and Coastal Sciences

Title of Dissertation: Intraspecific Variation in Salt Tolerance in Panicum Hemitomon, Spartina Patens, and Spartina Alterniflora: Population Differentiation and Investigations of Underlying Factors

Approved:

Irving A. Mendelssohn
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7-5-95